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Patent

GELS FOR ENCAPSULATION OF BIOLOGICAL MATERIALS

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August 1, 1995, which is a CIP of 07/958,870, filed 10/7/92,
15 which is a CIP of 07/870,540 (abandoned), filed 4/20/92; this is
also a CIP of 08/379,848, filed 1/27/95, which is a continuation
of 08/022,687 filed 3/1/93 and issued as U.S.P.N. 5,410,016,
which is a CIP of 07/843,485, filed 2/28/92 (abandoned); this is
also a CIP of 08/336,393, filed 11/10/94, which is a
20 continuation of 07/598,880, filed 10/15/90 (abandoned.)

BACKGROUND

Microencapsulation technology holds promise in many areas of medicine. For example, some important applications are treatment of diabetes (Goosen, M. F. A., et al. (1985) Biotechnology and Bioengineering, 27:146.), production of biologically important chemicals (Omata, T., et al. (1979) European J. Appl. Microbiol. Biotechnol., 6:207:215), evaluation of anti-human immuno-deficiency virus drugs (McMahon, J., et al., (1990) J. Nat. Cancer Inst., 82(22) 1761-1765),
25 encapsulation of hemoglobin for red blood cell substitutes, and controlled release of drugs. During encapsulation using prior methods, cells are often exposed to processing conditions which are potentially cytotoxic. These conditions include heat, organic solvents and

5 non-physiological pH which can kill or functionally impair cells.

Proteins are often exposed to conditions which are potentially denaturing and can result in loss of biological activity.

Further, even if cells survive processing conditions, the stringent requirements of encapsulating polymers for 10 biocompatibility, chemical stability, immunoprotection and resistance to cellular overgrowth, restrict the applicability of prior art methods. For example, the encapsulating method based on ionic crosslinking of alginate (a polyanion) with polylysine or polyornithine (polycation) (Goosen, M. F. A., et al. (1985) Biotechnology and Bioengineering, 27:146) offers relatively mild encapsulating conditions, but the long-term mechanical and chemical stability of such ionically crosslinked polymers remains doubtful. Moreover, these polymers when implanted *in vivo*, are susceptible to cellular overgrowth (McMahon, J., et al. (1990) J. Nat. Cancer Inst., 82(22) 1761-1765) which restricts the permeability of the microcapsule to nutrients, metabolites, and transport proteins from the surroundings. This has been seen to possibly lead to starvation and death of encapsulated islets of Langerhans cells (O'Shea, G. M. et al. (1986) Diabetes, 35:943-25 946).

Thus, there is a need for a relatively mild cell encapsulation method which offers control over properties of the encapsulating polymer. The membranes must be non-toxically produced in the presence of cells, with the qualities of being

5 permselective, chemically stable, and very highly biocompatible.
A similar need exists for the encapsulation of biological
materials other than cells and tissues.

Biocompatibility

Materials are considered biocompatible if the material
10 elicits either a reduced specific humoral or cellular immune
response or does not elicit a nonspecific foreign body response
that prevents the material from performing the intended function,
and if the material is not toxic upon ingestion or implantation.
The material also must not elicit a specific reaction such as
15 thrombosis if in contact with the blood.

Use of Gels in Biomaterials

Gels made of polymers which swell in water such as poly
(HEMA), water-insoluble polyacrylates, and agarose, have been
shown to be capable of encapsulating islet cells and other animal
20 tissue (Iwata, H., et al. (1989) Diabetes, 38:224-225; Lambert,
F. V., et al. (1984) Appl. Biochem. Biotech, 10:101-105).

However, these gels have undesirable mechanical properties.

Agarose forms a weak gel, and the polyacrylates must be
precipitated from organic solvents, thus increasing the potential
25 for cytotoxicity. Dupuy et al. ((1988) J. Biomed. Mater. Res.,
22:1061-1070) have reported the microencapsulation of islets by
polymerization of acrylamide to form polyacrylamide gels.
However, the polymerization process, if allowed to proceed
rapidly to completion, generates local heat and requires the

5 presence of toxic cross-linkers. This usually results in mechanically weak gels whose immunoprotective ability has not been established. Moreover, the presence of a low molecular weight monomer is required which itself is cytotoxic.

Microcapsules formed by the coacervation of alginate and poly(L-lysine) have been shown to be immunoprotective e.g., O'Shea, G. M. et al. (1986) Diabetes, 35:943-946. However, implantation for periods up to a week has resulted in severe fibrous overgrowth on these microcapsules (McMahon, J., et al. (1990) J. Nat. Cancer Inst., 82(22) 1761-1765; O'Shea, G. M. et al. (1986) Diabetes, 35:943-946).

Synthetic Biodegradable Polymers

The field of biodegradable polymers has developed rapidly since the synthesis and biodegradability of polylactic acid was first reported by Kulkarni et al., (1966) Arch. Surg., 93:839. Several other polymers are known to biodegrade, including polyanhydrides and polyorthoesters, which take advantage of labile backbone linkages, as reported by Domb et al., 1989 Macromolecules, 22:3200; Heller et al., 1990 BIODEGRADABLE POLYMERS AS DRUG DELIVERY SYSTEMS, Chasin, M. and Langer, R., Eds., Dekker, New York, 121-161. Since it is desirable to have polymers that degrade into naturally occurring materials, polyaminoacids have been synthesized, as reported by Miyake et al., (1974), for in vivo use. This was the basis for using polyesters (Holland et al., 1986 Controlled Release, 4:155-

5 180) of α -hydroxy acids (viz., lactic acid, glycolic acid), which remain the most widely used biodegradable materials for applications ranging from closure devices (sutures and staples) to drug delivery systems (U.S. Patent No. 4,741,337 to Smith et al.; Spilizewski et al., (1985) J. Control. Rel. 2:197-203).

10 The time required for a polymer to degrade can be tailored by selecting appropriate monomers. Differences in crystallinity also alter degradation rates. Due to the relatively hydrophobic nature of these polymers, actual mass loss only begins when the oligomeric fragments are small enough to be 15 water soluble. Hence, initial polymer molecular weight influences the degradation rate.

Degradable polymers containing water-soluble polymer elements have been described. Sawhney et al., (1990) J. Biomed. Mater. Res. 24:1397-1411, copolymerized lactide, glycolide and ϵ -caprolactone with PEG to increase its hydrophilicity and 20 degradation rate. U.S. Patent No. 4,716,203 to Casey et al. (1987) synthesized a PGA-PEG-PGA block copolymer, with PEG content ranging from 5-25% by mass. U.S. Patent No. 4,716,203 to Casey et al. (1987) also reports synthesis of PGA-PEG diblock 25 copolymers, again with PEG ranging from 5-25%. U.S. Patent No. 4,526,938 to Churchill et al. (1985) described noncrosslinked materials with MW in excess of 5,000, based on similar compositions with PEG; although these materials are not water soluble. Cohn et al. (1988) J. Biomed. Mater. Res. 22:993-1009

5 described PLA-PEG copolymers that swell in water up to 60%; these polymers also are not soluble in water, and are not crosslinked. These materials all use both water-soluble polymers and degradable polymers, and they are all insoluble in water, collectively swelling up to about 60%.

10 Degradable materials of biological origin are well known, for example, crosslinked gelatin. Hyaluronic acid has been crosslinked and used as a degradable swelling polymer for biomedical applications (U.S. Patent No. 4,987,744 to Della Valle et al., U.S. Patent 4,957,744 to Della Valle et al. (1991) Polym. Mater. Sci. Eng., 62:731-735).

Use of Biodegradable Materials for Controlled Drug Release

Most hydrophilic drugs are mechanically dispersed as suspensions within solutions of biodegradable polymers in organic solvents. Protein and enzyme molecular conformations are frequently different under these circumstances than they would be in aqueous media. An enzyme dispersed in such a hydrophobic matrix is usually present in an inactive conformation until it is released into the surrounding aqueous environment subsequent to polymer degradation. Additionally, some proteins may be irreversibly denatured by contact with organic solvents used in dispersing the protein within the polymer.

Use of PEO in Biomaterials

The use of poly(ethylene oxide) (PEO) to increase biocompatibility is well documented in the literature. The

5 presence of grafted PEO on the surface of bovine serum albumin
has been shown by Abuchowski, A. et al. ((1977) J. Biol. Chem.,
252:3578) to reduce immunogenicity in a rabbit and to increase
circulation times of exogenous proteins in animals. The
biocompatibility of algin-poly(L-lysine) microcapsules has been
10 significantly enhanced by incorporating a graft copolymer of PLL
and PEO on the microcapsule surface (Sawhney, et al.,
Biomaterials 13:863-870 (1991)).

The grafting of methoxy PEO onto polyacrylonitrile
surfaces was seen by Miyama et al. ((1988) *J. Appl. Polym. Sci.*,
15 35:115-125) to render the polyacrylonitrile surface relatively
non-thrombogenic. Nagoaka et al. (*Polymers as Biomaterials*,
Shalaby, S. W. ed., Plenum Press, New York) synthesized a graft
copolymer of methacrylates with PEO and found the resulting
polymer to be highly non-thrombogenic. Desai and Hubbell have
20 immobilized PEO on poly(ethylene terephthalate) surfaces by
forming a physical interpenetrating network (Desai et al., (1992)
Macromolecules 25:226); they have shown these surfaces to be
highly resistant to thrombosis (Desai et al., (1991) *Biomaterials*,
12:144) and to both mammalian and bacterial cell growth (Desai,
25 et al., submitted).

PEO is a unique polymer in terms of structure. The PEO
chain is highly water soluble and highly flexible. PEO chains
have an extremely high motility in water and are essentially non-
ionic in structure. The synthesis and characterization of PEO

5 derivatives which can be used for attachment of PEO to various surfaces, proteins, drugs etc. has been reviewed (Harris, (1985) JNS-Rev. Macromol. Chem. Phys., C25:325-373). Other polymers are also water soluble and non-ionic, such as poly(N-vinyl pyrrolidinone) and poly(ethyl oxazoline). These have been used
10 to reduce interaction of cells with tissues. Desai et al. (1991) Biomaterials, 12:144. Water soluble ionic polymers, such as hyaluronic acid, have also been used to reduce cell adhesion to surfaces and can similarly be used.

Electron beam cross-linking has been used to synthesize
15 PEO hydrogels, and these biomaterials have been reported to be non-thrombogenic (Sun, et al., (1987) Polymer Prepr., 28:292-294; Dennison, H.A., (1986) Ph.D. Thesis. Massachusetts Institute of Technology). However, use of an electron beam precludes the presence of any living tissue due to the sterilizing effect of
20 this radiation. Also, the networks produced are difficult to characterize due to the non-specific cross-linking induced by the electron beam.

Photopolymerizable PEG diacrylates have been used to entrap yeast cells for fermentation and chemical conversion
25 (Kimura et al. (1981) Eur. J. Appl. Microbiol. Biotechnol., 11:78-80; Omata et al., (1981) Eur. J. Appl. Microbial. Biotechnol., 11:199-204; Okada et al. (1987) Appl. Microbiol. Biotechnol., 26:112-116). Other methods for encapsulation of cells within materials photopolymerizable with short wavelength ultraviolet

5 radiation have been used with microbial cells (Tanaka, et al.,
(1977) Eur. J. Biochem, 80:193-197; Omata, et al., (1979)
European J. Appl. Microbiol. Biotechnol., 6:207-215; Omata, et
al., (1979) Eur. J. Appl. Microbiol. Biotechnol. 8:143-155; Chun,
et al., (1981) J. Gen. App. Microbiol., 27:505-509; Fukui, et al.,
10 (1976) Febs Letters, 66:2; Fukui, et al., (1984) Advances in
Biochemical Engineering and Biotechnology, 29:1-33). However,
yeast cells and some microbial cells are much hardier and
resistant to adverse environments, elevated temperatures, and
short wavelength ultraviolet radiation than mammalian cells and
15 human tissues.

There are several problems with these methods,
including the use of methods and/or materials which are
thrombogenic or unstable *in vivo*, or require polymerization
conditions which tend to destroy living mammalian tissue or
20 biologically active molecules, for example, short wavelength
ultraviolet radiation. In order to encapsulate living tissue for
implantation in a human or other mammalian subject, the
polymerization conditions must not destroy the living tissue, and
the resulting polymer-coated cells must be biocompatible.

25 There is also a need to encapsulate materials within a
very thin layer of material that is permeable to nutrients and
gases, yet strong and non-immunogenic. For example, for
transplantation of islets of Langerhans, the islets, which have a
diameter of 100 to 200 microns, have in the past been

5 encapsulated within microspheres that have a diameter of 400 to
1000 microns. This large diameter can result in slowed diffusion
of nutritional molecules and large transplantation volumes.

In summary, there is a need for materials, and methods
of use thereof, which can be used to encapsulate cells and
10 tissues or biologically active molecules which are biocompatible,
do not elicit specific or non-specific immune responses, and
which can be polymerized in contact with living cells or tissue
without injuring or killing the cells, within a very short time
frame, and in a very thin layer. An important aspect of the use
15 of these materials *in vivo* is that they must be polymerizable
within the time of a short surgical procedure or before the
material to be encapsulated disperses, is damaged or dies.

It is therefore an object of the present invention to
provide a polymeric material that can be polymerized in contact
20 with living cells and tissues, and in a very short time period.

It is a further object of the present invention to
provide a polymeric material which is biocompatible and resistant
to degradation for a specific time period.

It is a still further object of the present invention
25 to provide a polymeric material which is permeable to nutrients
and gases yet can protect cells and tissues from *in vivo* attack
by other cells.

5 It is yet a further object of the present invention to provide implantable biodegradable materials and biodegradable materials for encapsulation of cells and tissue.

It is another object of the present invention to provide biogels which can be both ionically and covalently 10 crosslinked.

It is an additional object of the present invention to provide a crosslinked biocompatible material which has at least one ionically crosslinked component and at least one covalently crosslinked component.

15 SUMMARY OF THE INVENTION

This invention provides novel methods for the formation of biocompatible membranes around biological materials using photopolymerization of water soluble molecules. The membranes can be used as a covering to encapsulate biological materials or 20 biomedical devices, as a "glue" to cause more than one biological substance to adhere together, or as carriers for biologically active species.

Several methods for forming these membranes are provided. Each of these methods utilizes a polymerization system 25 containing water-soluble macromers, species which are at once polymers and macromolecules capable of further polymerization. The macromers are polymerized using a photoinitiator (such as a dye), optionally a cocatalyst, optionally an accelerator, and

5 radiation in the form of visible or long wavelength UV light.

The reaction occurs either by suspension polymerization or by interfacial polymerization. The polymer membrane can be formed directly on the surface of the biological material, or it can be formed on material which is already encapsulated.

10 The macromers, which are water soluble or substantially water soluble, are too large to diffuse into the cells to be coated. Examples of macromers include highly biocompatible PEG hydrogels, which can be rapidly formed in the presence or absence of oxygen, without use of toxic polymerization initiators, at 15 room or physiological temperatures, and at physiological pH.

Some macromers of this invention include at least one water soluble region, at least one region which is biodegradable, usually by hydrolysis, and at least two free radical-polymerizable regions. The regions can, in some embodiments, be 20 both water soluble and biodegradable.

Ultrathin membranes can be formed by the methods described herein. These ultrathin membranes allow for optimal diffusion of nutrient and bioregulator molecules across the membrane, and great flexibility in the shape of the membrane. 25 Such thin membranes produce encapsulated material with optimal economy of volume. Biological material thus coated can be packed into a relatively small space without interference from bulky membranes.

5 The thickness and pore size of membranes formed can be
varied. This variability allows for "perm-selectivity" --
membranes can be adjusted to the desired degree of porosity,
allowing only preferred molecules to permeate the membrane, while
acting as a barrier against larger undesired molecules. Thus,
10 the membranes are immunoprotective in that they prevent the
transfer of antibodies or cells of the immune system.

When the encapsulated biological material is cellular
in nature, the absence of small monomers in the polymerization
solution prevents the diffusion of toxic molecules into the cell.
15 In this manner the present invention provides a polymerization
system which is more biocompatible than any available in the
prior art.

Additionally, the polymerization method can utilize
short bursts of visible or long wavelength UV light which is
20 nontoxic to biological material. Bioincompatible polymerization
initiators employed in the prior art are also eliminated.

According to the present invention, membrane formation
occurs under physiological conditions. Thus, no damage is done
to the enclosed biological material due to harsh pH, temperature,
25 or ionic conditions.

Because the membrane adheres to the biological material, the membrane can be used as an adhesive to fasten more than one biological substance together. The macromers are polymerized in the presence of these substances which are in close proximity.

5 The membrane forms in the interstices, effectively gluing the substances together.

10 Additionally, utilizing the tendency of the membrane to adhere to biological material, a membrane can be formed around or on a biologically active substance to act as a carrier for that substance.

BRIEF DESCRIPTION OF THE FIGURES

15 Figure 1 shows schematically illustrated macromers of the present invention where _____ is a water soluble core such as PEG; ----- is a hydrolyzably degradable extension such as a polyglycolide; ===== is a polymerizable end cap or side chain such as an acrylate; and ----- is a water-soluble and hydrolyzable portion such as a hyaluronate.

20 Figure 2A is a schematic of dye-initiated polymerization of a PEG layer around crosslinked alginate microspheres.

25 Figure 2B is a photomicrograph of the alginate/poly(L-lysine) microspheres containing human islets of Langerhans coated with a PEG 18.5K tetraacrylate hydrogel using the dye binding method depicted in Figure 2A.

Figure 3 is a schematic of photopolymerization of a PEG coating on alginate-poly(L-lysine) microspheres suspended in mineral oil.

5 Figure 4 is a photomicrograph of Islets of Langerhans isolated from a human pancreas encapsulated in a PEG 18.5K tetraacrylate hydrogel.

Figure 5 is a schematic representation of coextrusion apparatus used for microencapsulation using laser polymerization

10 Figure 6 is a photomicrograph of microspheres produced by laser polymerization of PEG 400 diacrylate around cells.

Figure 7A is a photomicrograph of alginate-PLL microspheres recovered after 4 days following implantation i.p. in mice.

15 Figure 7B is a photomicrograph of Alginate-PLL microspheres coated with a PEG 18.5K Da tetraacrylate, using the dye diffusion method depicted in Figure 1.

20 Figures 8A-F is a graph of the number of cells versus gel composition, for the unattached cells obtained from lavage of the peritoneal cavity in mice with different PEO overcoat gel compositions: a - 18.5k; b - 10% 0.5k, 90% 18.5k; c - 50% 18.5k, 50% 0.4k; d - 10% 0.4k, 90% 35k; e - 50% 0.4k, 50% 35k; and f - alginate-poly(L-lysine) control.

25 Figure 9 is a graph of the % protein released versus time in minutes, for diffusion of bovine serum albumin (open squares), human IgG (triangles) and human fibrinogen (closed squares) through a PEO 18.5K-tetraacrylate gel.

Figure 10 is a graph of the % diffusion of bovine serum albumin over time in minutes through PEO 400 diacrylate (open squares) and PEG 18.5K-tetracrylate (triangles) gels.

5 Figure 11A is a graph of the length in mm of gel produced by argon ion laser induced polymerization versus log (time) (ms) of trimethylolpropane using an amine and ethyl eosin initiation system.

10 Figure 11B is a photomicrograph of the spikes formed as a result of laser irradiation of ethoxylated trimethylol propane triacrylate for durations of 67 ms, 125 ms, 250 ms, 500 ms, and 1 sec.

15 Figure 12A is a photomicrograph of human foreskin fibroblasts cultured for 6 h on a glass coverslip coated with PEG 18.5K-tetraacrylate gel.

Figure 12B is a photomicrograph of human foreskin fibroblasts cultured for 6 h on a glass that was not coated with PEG.

20 Figure 13 is a photomicrograph of PEG 18.5K-tetraacrylate microspherical gels, implanted in mice, and explanted after 4 days, showing very little fibrous overgrowth.

Figures 14A-C are creep curves for PEG diacrylate and tetraacrylate gels; test and recovery loads are given below the abscissa: A - 1k; B - 6K; and C - 18.5K PEG gels.

25 Figure 15 shows the degree of photopolymerization (dp) calculated and found by NMR.

Figure 16A shows Human foreskin fibroblasts cultured for six hours on glass coverslips coated with PEG 18.5K-glycolide diacrylate (18.5KG).

5 Figure 16B shows Human foreskin fibroblasts cultured for six hours on glass coverslips not coated with PEG.

Figure 17A shows the release of BSA from a PEG 1K (1000 molecular weight PEG) glycolide diacrylate with glycolide extensions (1 KG) hydrogel into PBS.

10 Figure 17B shows release of lysozyme from PEG 18.5K-DL-lactide tetraacrylate (18.5KL) into PBS.

Figure 18A shows release of active recombinant tPA from a PEG 1K lactide diacrylate (1KL) hydrogel.

15 Figure 18B shows release of active recombinant t-PA from PEG 4K glycolide diacrylate (4KG) hydrogel.

Figure 18C shows release of active recombinant tPA from a PEG 18.5K-glycolide diacrylate (18.5KG) hydrogel into PBS.

20 Figure 19A is a superior view of rabbit uterine horn used as a control. Distorted horn anatomy with 66% adhesions is evident. The horns are folded upon themselves.

Figure 19B is a superior view of rabbit uterine horn treated with a photopolymerized biodegradable hydrogel, PEG 18.5KL. Horn anatomy is normal, with no adhesion bands visible.

25 Figure 20A is an environmental scanning electron micrograph (ESEM) of an untreated blood vessel following trauma.

Figure 20B is an ESEM of a polymer coated blood vessel following trauma.

DETAILED DESCRIPTION

By a variety of methods, this invention provides a means for creating biocompatible membranes of varying thickness on the surface of a variety of biological materials. The polymerization occurs by a free-radical reaction, causing a 10 "macromer" with at least two ethylenically unsaturated moieties to form a crosslinked polymer. The components of this reaction are:

- (1) a photoinitiator, preferably eosin dye;
- (2) a "macromer," preferably polyethylene glycol (PEG) diacrylate, m.w. 18.5 kD. This component is at once a polymer and a macromolecule capable of further polymerization;
- (3) optionally a cocatalyst, preferably triethanolamine; and
- (4) optionally, an accelerator.

These components are mixed in varying combinations, and the mixture is exposed to longwave UV or visible light ("radiation"), preferably of wavelength 350-700 nm, most preferred at 365-514 nm, to initiate polymerization. A network is formed as the 25 macromers polymerize in a variety of directions.

5 POLYMERIZATION

Four methods are used to effect polymerization to form biocompatible membranes. These are referred to below as the "bulk suspension polymerization" method, the "microcapsule suspension polymerization" method, the "microcapsule interfacial polymerization" method, and the "direct interfacial polymerization" method. They utilize either suspension or interfacial polymerization techniques on either coated or uncoated biological material.

15 1. BULK SUSPENSION POLYMERIZATION METHOD

In this embodiment of the invention the core biological material is mixed in an aqueous macromer solution (composed of the macromer, cocatalyst and optionally an accelerator) with the photoinitiator. Small globular geometric structures such as spheres, ovoids, or oblongs are formed, preferably either by coextrusion of the aqueous solution with air or with a non-miscible substance such as oil, preferably mineral oil, or by agitation of the aqueous phase in contact with a non-miscible phase such as an oil phase to form small droplets. The macromer in the globules is then polymerized when exposed to radiation. Because the macromer and initiator are confined to the globules, the structure resulting from polymerization is a capsule in which the biological material is enclosed. This is a "suspension polymerization" whereby the entire aqueous portion of the globule

5 polymerizes to form a thick membrane around the cellular material.

2. MICROCAPSULE SUSPENSION POLYMERIZATION METHOD

This embodiment of the invention employs microencapsulated material as a core about which the macromer is polymerized 10 in a suspension polymerization reaction. The biological material is first encapsulated, such as in an alginate microcapsules. The microcapsule is then mixed as in the first embodiment with the macromer solution and the photoinitiator, and then polymerized by radiation. In the event an ionically crosslinkable material such 15 as alginate is used for the first encapsulation, this results in a biocompatible material which is both ionically crosslinked and covalently crosslinked.

This method takes advantage of the extreme hydrophilicity of PEG macromer, and is especially suited for use 20 with hydrogel microcapsules such as alginate-poly(L-lysine). The microsphere is swollen in water. When a macromer solution (with the initiating system) is forced to phase separate in a hydrophobic medium, such as mineral oil, the PEG macromer solution prefers to stay on the hydrophilic surface of the 25 alginate microcapsule. When this suspension is irradiated, the PEG macromer undergoes polymerization and gelation, forming a thin layer of polymeric, water insoluble gel around the microsphere. Agarose beads have been used in an analogous way by Gin et al. (1987) J. Microencapsulation, 4:239-242 as scaffolds to

5 carry out polymerization of acrylamide. However, that method is limited by potential toxicity associated with the use of a low molecular weight monomer, as opposed to the macromeric precursors of the present invention.

This technique preferably involves coextrusion of the
10 microcapsule in a solution of macromer and photoinitiator, the solution being in contact with air or a liquid which is non-miscible with water, to form droplets which fall to a container such as a petri dish containing a solution such as mineral oil in which the droplets are not miscible. The non-miscible liquid is
15 chosen for its ability to maintain droplet formation.

Additionally, if the membrane-encapsulated material is to be injected or implanted in an animal, any residue should be non-toxic and non-immunogenic. Mineral oil is a preferred non-miscible liquid.

20 On the petri dish the droplets are exposed to radiation which causes polymerization. This coextrusion technique results in a crosslinked polymer coat of greater than 50 microns thickness. Alternatively, the microcapsules may be suspended in a solution of macromer and photoinitiator which is agitated in
25 contact with a non-miscible phase such as an oil phase. The emulsion which results is irradiated to form a polymer coat, again of greater than 50 microns thickness.

5 3. MICROCAPSULE INTERFACIAL POLYMERIZATION METHOD

In this embodiment, the biological material is also microencapsulated as in the previous method. However, rather than suspension polymerization, interfacial polymerization is utilized to form the membrane. This involves coating the 10 microcapsule with photoinitiator, suspending the microcapsule in the macromer solution, and immediately irradiating. By this technique a thin polymer coat, of less than 50 microns thickness, is formed about the microcapsule, because the photoinitiator is present only at the microcapsule surface and is given 15 insufficient time to diffuse far into the macromer solution. As a result, the initiator is present in only a thin shell of the aqueous solution, causing a thin layer to be polymerized.

When the microcapsules are in contact with dye solution, the dye penetrates into the inner core of the 20 microcapsule as well as adsorbing to the surface. When such a microcapsule is put into a solution containing a macromer and, optionally, a cocatalyst such as triethanolamine, and exposed to laser light, initially all the essential components of the reaction are present only at and just inside the interface of 25 microcapsule and macromer solution. Hence, the polymerization and gelation (if multifunctional macromer is used) initially takes place only at the interface, just beneath it, and just beyond it. If left for longer periods of time, the dye starts

5 diffusing from the inner core of the microsphere into the solution; similarly, macromers start diffusing inside the core.

Polymerization and subsequent gelation are very rapid (typical gelation times are 100 ms) (Fouassier, et al., (1985) J. Polym. Sci., Polym. Chem. Ed., 23:569; Chesneau, et al., (1985) Die Ange. Makromol. Chemie, 135:41, (1988) Makromol. Chem., Rapid Commun., 9:223). Because diffusion is a much slower process than polymerization, not the entire macromer solution is polymerized or gelled. Essentially the reaction is restricted to the near surface only. The dye, being a smaller molecule and being weakly bound to the capsule materials, keeps diffusing out of the microsphere. If this diffusion occurs under laser irradiation, then dye at the interface is used continuously to form a thicker gel layer. The thickness of the coating can thus be directed by controlling the reaction conditions.

20 A schematic representation of this process is shown in Figure 2A. The amount, thickness or size and rigidity of the gel formed will depend on the size and intensity of the beam, time of exposure, initiator, macromer molecular weight, and macromer concentration (see below). Alginate/PLL microspheres containing 25 islets coated by this technique are shown in Figure 2B.

4. DIRECT INTERFACIAL POLYMERIZATION METHOD

The fourth embodiment of this invention utilizes interfacial polymerization to form a membrane directly on the surface of the biological material. This results in the smallest

5 capsules and thus achieves optimal economy of volume. Tissue is directly coated with photoinitiator, emersed in the macromer solution, and immediately irradiated. This technique results in a thin polymer coat surrounding the tissue since there is no space taken up by a microcapsule, and the photoinitiator is again 10 present only in a thin shell of the macromer solution.

USE AS AN ADHESIVE

It is usually difficult to get good adhesion between polymers of greatly different physicochemical properties. The concept of a surface physical interpenetrating network was presented by Desai and Hubbel (Desai et al. (1992) Macromolecules 15 25:226). This approach to incorporating into the surface of one polymer a complete coating of a polymer of considerably different properties involved swelling the surface of the polymer to be modified (base polymer) in a mutual solvent, or a swelling 20 solvent, for the base polymer and for the polymer to be incorporated (penetrant polymer). The penetrant polymer diffused into the surface of the base polymer. This interface was stabilized by rapidly precipitating or deswelling the surface by placing the base polymer in a nonsolvent bath. This resulted in 25 entanglement of the penetrant polymer within the matrix of the base polymer at its surface in a structure that was called a surface physical interpenetrating network.

5 This approach can be improved upon by photopolymerizing
the penetrant polymer upon the surface of the base polymer in the
swollen state. This results in much enhanced stability over that
of the previous approach and in the enhancement of biological
responses to these materials. The penetrant may be chemically
10 modified to be a prepolymer (macromer), i.e. capable of being
polymerized itself. This polymerization can be initiated
thermally or by exposure to visible, ultraviolet, infrared, gamma
ray, or electron beam irradiation, or to plasma conditions. In
the case of the relatively nonspecific gamma ray or electron beam
15 radiation reaction, chemical incorporation of particularly
reactive sites may not be necessary.

TOP SECRET//EYES ONLY

Polyethylene glycol (PEG) is a particularly useful
penetrant polymer for biomedical applications where the lack of
cell adhesion is desired. The previous work had demonstrated an
optimal performance at a molecular weight of 18,500 D without
chemical crosslinking. PEG prepolymers can be readily formed by
acrylation of the hydroxyl groups at its termini or elsewhere
within the chain. These prepolymers can be readily polymerized
by the above described radiation methods. Photoinitiated
20 polymerization of these prepolymers is particularly convenient
and rapid. There are a variety of visible light initiated and
ultraviolet light initiated reactions that are initiated by light
absorption by specific photochemically reactive dyes, described
25 elsewhere herein. This same approach can be used for biomedical

5 purposes with other water-soluble polymers, such as poly(N-vinyl
pyrrolidinone), poly(N-isopropyl acrylamide), poly(ethyl
oxazoline) and many others.

10 Additionally, it is usually difficult to obtain
adhesives for wet surfaces and tissues. Water soluble
10 prepolymers, for example PEG diacrylates, can be used for this
purpose. When a water soluble polymer is placed in aqueous
solution upon a tissue, the polymer diffuses into the surface of
the tissue, within the protein and polysaccharide matrix upon the
tissue but not within the cells themselves. When the water
15 soluble polymer is a prepolymer and a visible, ultraviolet or
infrared photoinitiator is included, the polymer penetrant may be
exposed to the appropriate light to gel the polymer. In this
way, the polymer is crosslinked within and around the matrix of
the tissue in what is called an interpenetrating network. If the
20 prepolymer is placed in contact with two tissues and the
polymer is illuminated, then these two tissues are adhered
together by the intermediate polymer gel.

BIOLOGICAL MATERIALS

25 Due to the biocompatibility of the materials and
techniques involved, a wide variety of materials can be used in
conjunction with the present invention. For encapsulation, the
techniques can be used with mammalian tissue and/or cells, as
well as sub-cellular organelles and other isolated sub-cellular

5 components. The membranes can be crafted to meet the perm-selectivity needs of the biological material enclosed. Cells which are to be used to produce desired products such as proteins are optimally encapsulated by this invention.

Examples of cells which can be encapsulated are primary cultures as well as established cell lines, including transformed cells. These include but are not limited to pancreatic islet cells, human foreskin fibroblasts, Chinese hamster ovary cells, beta cell insulomas, lymphoblastic leukemia cells, mouse 3T3 fibroblasts, dopamine secreting ventral mesencephalon cells, neuroblastoid cells, adrenal medulla cells, and T-cells. As can be seen from this partial list, cells of all types, including dermal, neural, blood, organ, muscle, glandular, reproductive, and immune system cells can be encapsulated successfully by this method. Additionally, proteins (such as hemoglobin), polysaccharides, oligonucleotides, enzymes (such as adenosine deaminase), enzyme systems, bacteria, microbes, vitamins, cofactors, blood clotting factors, drugs (such as TPA, streptokinase or heparin), antigens for immunization, hormones, and retroviruses for gene therapy can be encapsulated by these techniques.

The biological material can be first enclosed in a structure such as a polysaccharide gel. (Lim, U.S.P.N. 4,352,883; Lim, U.S.P.N. 4,391909; Lim, U.S.P.N. 4,409,331; Tsang, et al., U.S.P.N. 4,663,286; Goosen et al., U.S.P.N.

5 4,673,556; Goosen et al., U.S.P.N. 4,689,293; Goosen et al.,
U.S.P.N. 4,806,355; Rha et al., U.S.P.N. 4,744,933; Rha et al.,
U.S.P.N. 4,749,620, incorporated herein by reference.) Such gels
can provide additional structural protection to the material, as
well as a secondary level of perm-selectivity. If alginate is
10 used, it is preferred that the alginate be relatively high in α -
L-guluronic acid content. This "high G" content increases the
biocompatibility of the material. The alginate should be at
least 60% α -L-guluronic acid, and more preferably at least 70% α -
L-guluronic acid.

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15 MACROMERS

Polymerization via this invention utilizes macromers
rather than monomers as the building blocks. The macromers are
small polymers which are susceptible to polymerization into the
larger polymer membranes of this invention. Polymerization is
enabled because the macromers contain sites of unsaturation,
e.g., carbon-carbon double bond moieties, carbon-carbon triple
bond moieties, and the like, as well as sites of unsaturation
between carbon atoms and heteroatoms and between two heteroatoms.

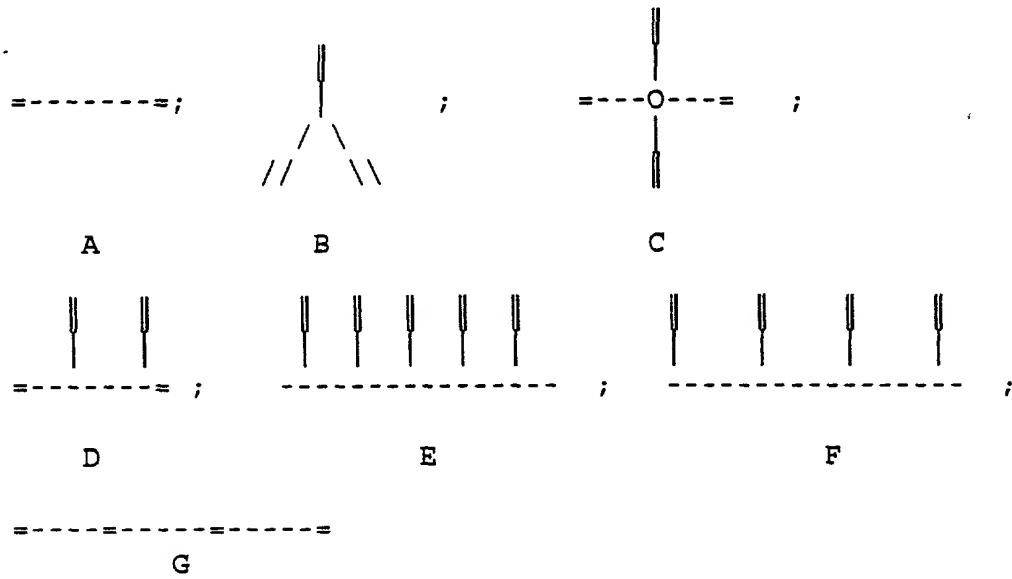
20 Examples of carbon-carbon double bonds useful in this invention
include acrylate, methacrylate, ethacrylate, 2-phenyl acrylate,
2-chloro acrylate, 2-bromo acrylate, itaconate, acrylamide,
methacrylamide, and styrene groups. The macromers are water

5 soluble compounds and are non-toxic to biological material before
and after polymerization.

A wide variety of substantially water soluble polymers
exist, some of which are illustrated schematically below.

(—) represents a substantially water soluble region of the
10 polymer, and (=) represents a free radical polymerizable species.

Examples include:



15 Examples of A include PEG diacrylate, from a PEG diol;
20 Examples of B include PEG triacrylate, formed from a PEG triol; of C
include PEG-cyclodextrin tetraacrylate, formed by grafting PEG to
a cyclodextrin central ring, and further acrylating; of D include
PEG tetraacrylate, formed by grafting two PEG diols to a bis
epoxide and further acrylating; of E include hyaluronic acid
25 methacrylate, formed by acrylating many sites on a hyaluronic
acid chain; of F include PEG-hyaluronic acid-methacrylate,
30

5 formed by grafting PEG to hyaluronic acid and further acrylating; of G include PEG-unsaturated diacid ester formed by esterifying a PEG diol with an unsaturated diacid.

10 Polysaccharides include, for example, alginate (preferably high-G alginate), hyaluronic acid, chondroitin sulfate, dextran, dextran sulfate, heparin, heparin sulfate, heparan sulfate, chitosan, gellan gum, xanthan gum, guar gum, and K-carrageenan. Proteins, for example, include gelatin, collagen, elastin and albumin, whether produced from natural or recombinant sources. In the event that a substance such as alginate or hyaluronic acid is used, the resulting macromers will be both ionically crosslinkable and covalently crosslinkable.

15 Photopolymerizable substituents preferably include acrylates, diacrylates, oligoacrylates, dimethacrylates, or oligomethacrylates, and other biologically acceptable photopolymerizable groups.

Synthetic Polymeric Macromers

20 The water-soluble macromer may be derived from water-soluble polymers including, but not limited to, poly(ethylene oxide) (PEO), poly(ethylene glycol) (PEG), poly(vinyl alcohol) (PVA), poly(vinylpyrrolidone) (PVP), poly(ethyloxazoline) (PEOX) polyaminoacids, pseudopolyamino acids, and polyethyloxazoline, as well as copolymers of these with each other or other water soluble polymers or water insoluble polymers, provided that the conjugate is water soluble. An example of a water soluble

5 conjugate is a block copolymer of polyethylene glycol and polypropylene oxide, commercially available as a Pluronic™ surfactant.

Polysaccharide Macromers

10 Polysaccharides such as alginate (preferably high-G alginate), hyaluronic acid, chondroitin sulfate, dextran, dextran sulfate, heparin, heparin sulfate, heparan sulfate, chitosan, gellan gum, xanthan gum, guar gum, water soluble cellulose derivatives, and carrageenan, which are linked by reaction with hydroxyls or amines on the polysaccharides can also be used to form the macromer solution. As noted above, in the event that alginate, hyaluronic acid, or other ionically gellable materials are used, the macromers are both ionically crosslinkable and covalently crosslinkable.

Protein Macromers

20 Proteins such as gelatin, collagen, elastin, zein, and albumin, whether produced from natural or recombinant sources, which are made free-radical polymerization by the addition of carbon-carbon double or triple bond-containing moieties, including acrylate, diacrylate, methacrylate, ethacrylate, 2-phenyl acrylate, 2-chloro acrylate, 2-bromo acrylate, itaconate, oligoacrylate, dimethacrylate, oligomethacrylate, acrylamide, methacrylamide, styrene groups, and other biologically acceptable photopolymerizable groups, can also be used to form the macromer solution.

5 MACROMERS FOR BIODEGRADABLE GELS

In general terms, the macromers for biodegradable gels are polymers that are soluble in aqueous solutions, or nearly aqueous solutions, such as water with added dimethylsulfoxide. They have three components including a biodegradable region, 10 preferably hydrolyzable under *in vivo* conditions, a water soluble region, and at least two polymerizable regions. Examples of these structures are shown in Figure 1.

Structure A in Figure 1 shows a macromer having a water soluble region (_____), a water soluble and degradable component (-----) appended to one another. Each has a polymerizable end cap (=====). Structure B shows a major water soluble component or core region (_____) extended at either end by a degradable or hydrolyzable component (-----) and terminated by, at either end, a polymerizable component (=====). Structure C shows a central degradable or hydrolyzable component (-----) bound to a water soluble component (_____) capped at either end by a polymerizable component (=====). Structure D shows a central water soluble component (_____) with numerous branches of hydrolyzable components (-----), each hydrolyzable component 20 being capped with a polymerizable component (=====). Structure E shows a central biodegradable, hydrolyzable component (-----) with three water soluble branches (_____), each water soluble branch being capped by a polymerizable component (=====). Structure F shows a long central water soluble and hydrolyzable

5 component (-----), each end being capped by a polymerizable component (=====). Structure G shows a central water soluble and hydrolyzable component (-----) capped at both ends by a hydrolyzable component (~~~~~), each hydrolyzable component being capped by a polymerizable component (=====). Structure H
10 shows a central water soluble and degradable or hydrolyzable component (-----) with end caps or branches of a polymerizable component (=====). Structure I shows a central water soluble component (_____) in circular form with water soluble branches extended by a hydrolyzable component (~~~~~) capped by a polymerizable component (=====). Lastly, Structure J in Figure
15 1 shows a circular water soluble core component (_____) with degradable branches (~~~~~), each being capped by a polymerizable component (-----).

0
15
20
25

The various structures shown in Figure 1 are exemplary only. Those skilled in the art will understand many other possible combinations which could be utilized for the purposes of the present invention.

Used herein is the term "at least substantially water soluble." This is indicative that the solubility should be at least about 1 g/100 ml of aqueous solution or in aqueous solution containing small amounts of organic solvent, such as dimethylsulfoxide. By the term "polymerizable" is meant that the regions have the capacity to form additional covalent bonds resulting in macromer interlinking, for example, carbon-carbon

5 double bonds of acrylate-type molecules. Such polymerization is
characteristically initiated by free-radical formation, for
example, resulting from photon absorption of certain dyes and
chemical compounds to ultimately produce free-radicals.

In a preferred embodiment, a hydrogel begins with a
10 biodegradable, polymerizable, macromer including a core, an
extension on each end of the core, and an end cap on each
extension. The core is a hydrophilic polymer or oligomer; each
extension is a biodegradable polymer or oligomer; and each end
cap is an oligomer, dimer or monomer capable of cross-linking the
macromers. In a particularly preferred embodiment, the core
includes hydrophilic poly(ethylene glycol) oligomers of molecular
weight between about 400 and 30,000 Da; each extension includes
biodegradable poly (α -hydroxy acid) oligomers of molecular weight
between about 200 and 1200 Da; and each end cap includes an
acrylate-type monomer or oligomer (i.e., containing carbon-carbon
double bonds) of molecular weight between about 50 and 200 Da
which are capable of cross-linking and polymerization between
copolymers. More specifically, a preferred embodiment
incorporates a core consisting of poly(ethylene glycol) oligomers
25 of molecular weight between about 8,000 and 10,000 Da; extensions
consisting of poly(lactic acid) oligomers of molecular weight
about 250 Da; and end caps consisting acrylate moieties of about
100 Da molecular weight.

5 Those skilled in the art will recognize that oligomers
of the core, extensions and end caps may have uniform
compositions or may be combinations of relatively short chains or
individual species which confer specifically desired properties
on the final hydrogel while retaining the specified overall
10 characteristics of each section of the macromer. The lengths of
oligomers referred to herein may vary from two mers to many, the
term being used to distinguish subsections or components of the
macromer from the complete entity.

15 Water Soluble Regions

In preferred embodiments, the core water soluble region
can consist of poly(ethylene glycol), poly(ethylene oxide),
poly(vinyl alcohol), poly(vinylpyrrolidone),
poly(ethyloxazoline), poly(ethylene oxide)-co-
poly(propyleneoxide) block copolymers, polysaccharides or
carbohydrates such as hyaluronic acid, dextran, heparan sulfate,
chondroitin sulfate, heparin, or alginate (preferably high-G
alginate), proteins such as gelatin, collagen, albumin,
ovalbumin, or polyamino acids.

20 Biodegradable Regions

25 The biodegradable region is preferably hydrolyzable
under *in vivo* conditions. For example, hydrolyzable group may be
polymers and oligomers of glycolide, lactide, ϵ -caprolactone,
other hydroxy acids, and other biologically degradable polymers
that yield materials that are non-toxic or present as normal

metabolites in the body. Preferred poly(α -hydroxy acid)s are poly(glycolic acid), poly(DL-lactic acid) and poly(L-lactic acid). Other useful materials include poly(amino acids), poly(anhydrides), poly(orthoesters), poly(phosphazines) and poly(phosphoesters). Polylactones such as poly(ϵ -caprolactone), poly(ϵ -caprolactone), poly(δ -valerolactone) and poly(gamma-butylolactone), for example, are also useful. The biodegradable regions may have a degree of polymerization ranging from one up to values that would yield a product that was not substantially water soluble. Thus, monomeric, dimeric, trimeric, oligomeric, and polymeric regions may be used.

Biodegradable regions can be constructed from polymers or monomers using linkages susceptible to biodegradation, such as ester, peptide, anhydride, orthoester, phosphazine and phosphoester bonds.

Polymerizable Regions

The polymerizable regions are preferably polymerizable by photoinitiation by free radical generation, most preferably in the visible or long wavelength ultraviolet radiation. The preferred polymerizable regions are acrylates, diacrylates, 25 oligoacrylates, methacrylates, dimethacrylates, oligomethacrylates, or other biologically acceptable photopolymerizable groups.

Other initiation chemistries may be used besides photoinitiation. These include, for example, water and amine

5 initiation schemes with isocyanate or isothiocyanate containing
macromers used as the polymerizable regions.

MACROMER SIZE

These macromers can vary in molecular weight from 0.2-
100 kD, depending on the use. The degree of polymerization, and
10 the size of the starting macromers, directly affect the porosity
of the resulting membrane. Thus, the size of the macromers are
selected according to the permeability needs of the membrane.

For purposes of encapsulating cells and tissue in a manner which
prevents the passage of antibodies across the membrane but allows
15 passage of nutrients essential for cellular metabolism, the
preferred starting macromer size is in the range of 10 kD to 18.5
kD, with the most preferred being around 18.5 kD. Smaller
macromers result in polymer membranes of a higher density with
smaller pores.

PHOTOINITIATING DYES

The photoinitiating dyes capture light energy and
initiate polymerization of the macromers. Any dye can be used
which absorbs light having frequency between 320 nm and 900 nm,
can form free radicals, is at least partially water soluble, and
25 is non-toxic to the biological material at the concentration used
for polymerization. Examples of suitable dyes are ethyl eosin,
eosin Y, fluorescein, 2,2-dimethoxy,2-phenylacetophenone, 2-

5 methoxy, 2-phenylacetophenone, camphorquinone, rose bengal, methylene blue, erythrosin, phloxime, thionine, riboflavin and methylene green. The preferred initiator dye is ethyl eosin due to its spectral properties in aqueous solution.

COCATALYST

10 The cocatalyst is a nitrogen based compound capable of stimulating the free radical reaction. Primary, secondary, tertiary or quaternary amines are suitable cocatalysts, as are any nitrogen atom containing electron-rich molecules.

15 Cocatalysts include, but are not limited to, triethanolamine, triethylamine, ethanolamine, N-methyl diethanolamine, N,N-dimethyl benzylamine, dibenzyl amine, N-benzyl ethanolamine, N-isopropyl benzylamine, tetramethyl ethylenediamine, potassium persulfate, tetramethyl ethylenediamine, lysine, ornithine, histidine and arginine.

20 RADIATION WAVELENGTH

The radiation used to initiate the polymerization is either longwave UV or visible light, with a wavelength in the range of 320-900 nm. Preferably, light in the range of 350-700 nm, and even more preferred in the range of 365-514 nm, is used.

25 This light can be provided by any appropriate source able to generate the desired radiation, such as a mercury lamp, longwave UV lamp, He-Ne laser, or an argon ion laser.

5 THICKNESS AND CONFORMATION OF POLYMER LAYER

Membrane thickness affects a variety of parameters, including perm-selectivity, rigidity, and size of the membrane.

In the interfacial polymerization method, the duration of the radiation can be varied to adjust the thickness of the polymer

10 membrane formed. This correlation between membrane thickness and duration of irradiation occurs because the photoinitiator

diffuses at a steady rate, with diffusion being a continuously occurring process. Thus, the longer the duration of irradiation,

the more photoinitiator will initiate polymerization in the

15 macromer mix, the more macromer will polymerize, and a thicker

coat will be formed. Additional factors which affect membrane

thickness are the number of reactive groups per macromer, the

concentration of accelerators in the macromer solution. This

technique allows the creation of very thin membranes because the

20 photoinitiator is first present in a very thin layer at the

surface of the biological material, and polymerization only

occurs where the photoinitiator is present.

The suspension polymerization method forms a somewhat thicker membrane than the interfacial polymerization method.

25 This is because polymerization occurs in the suspension method

throughout the macromer mix. The thickness of membranes formed

by the suspension method is determined in part by the viscosity

of the macromer solution, the concentration of the macromer in

that solution, the fluid mechanical environment of the suspension

5 and surface active agents in the suspension. These membranes vary in thickness from 50-300 microns. The shape of the structure formed by suspension polymerization can be controlled by shaping the reaction mix prior to polymerization. Spheres can be formed by emulsion with a non-miscible liquid such as oil, 10 coextrusion with such a liquid, or coextrusion with air. Cylinders may be formed by casting or extrusion, and slabs and discoidal shapes can be formed by casting. Additionally, the shape may be formed in relationship to an internal supporting structure such as a screening network of stable polymers (e.g. an 15 alginate gel, preferably high-G alginate, or a woven polymer fiber) or nontoxic metals.

The overall amount, thickness, and rigidity of the membrane formed depends on the interaction of several parameters, including the size and intensity of the radiation beam, duration 20 of exposure of the solution to the radiation, reactivity of the initiator selected, macromer molecular weight, and macromer concentration.

APPLICATIONS FOR THE MACROMERS

The invention can be used for a variety of purposes, 25 some of which are enumerated below, along with benefits which accrue from the use of the invention. Some additional purposes are illustrated by the Examples which follow.

5 a. Microencapsulating cells: more biocompatible, stronger,
more stable, better control of permselectivity, less
toxic conditions

10 b. Macroencapsulating cells: more biocompatible, stronger,
more stable, better control of permselectivity, less
toxic conditions, easier to incorporate internal or
external supporting structure

15 c. Microencapsulating or macroencapsulating other tissues,
with the same benefits

20 d. Microencapsulating or macroencapsulating pharmaceuticals:
more biocompatible, less damaging to the
pharmaceutical

25 e. Coating devices: ease of application, more
biocompatible

30 f. Coating microcapsules: more biocompatible, strengthens
them, ease of coating

35 g. Coating macrocapsules, microcapsules, microspheres and
macrospheres: more biocompatible, ease of coating

40 h. Coating tissues to alter adhesion of other tissues:
ease of coating, less toxicity to the tissues,
conformal coating versus nonconformal

45 i. Adhesive between two tissues: ease of adhesion,
rapidity of forming adhesive bond, less toxicity to
tissues

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5

Applications for the Biodegradable Macromers

Further, the biodegradable macromers can be used for the following purposes.

Prevention of Surgical Adhesions

A preferred application is a method of reducing formation of adhesions after a surgical procedure in a patient.

The method includes coating damaged tissue surfaces in a patient with an aqueous solution of a light-sensitive free-radical polymerization initiator and a macromer solution as described above. The coated tissue surfaces are exposed to light sufficient to polymerize the macromer. The light-sensitive free-radical polymerization initiator may be a single compound (e.g., 2,2-dimethoxy-2-phenyl acetophenone) or a combination of a dye and a cocatalyst (e.g., ethyl eosin and triethanol amine).

Controlled drug delivery

A second preferred application concerns a method of locally applying a biologically active substance to tissue surfaces of a patient. The method includes the steps of mixing a biologically active substance with an aqueous solution including a light-sensitive free-radical polymerization initiator and a macromer as described above to form a coating mixture. Tissue surfaces are coated with the coating mixture and exposed to light sufficient to polymerize the macromer. The biologically active substance can be any of a variety of materials, including proteins, carbohydrates, nucleic acids, and inorganic and organic

5 biologically active molecules. Specific examples include enzymes, antibiotics, antineoplastic agents, local anesthetics, hormones, antiangiogenic agents, antibodies, neurotransmitters, psychoactive drugs, drugs affecting reproductive organs, and oligonucleotides such as antisense oligonucleotides.

10 In a variation of the method for controlled drug delivery, the macromers are polymerized with the biologically active materials to form microspheres or nanoparticles containing the biologically active material. The macromer, photoinitiator, and agent to be encapsulated are mixed in an aqueous mixture. Particles of the mixture are formed using standard techniques, for example, by mixing in oil to form an emulsion, forming droplets in oil using a nozzle, or forming droplets in air using a nozzle. The suspension or droplets are irradiated with a light suitable for photopolymerization of the macromer.

15 20 Tissue Adhesives

Another use of the polymers is in a method for adhering tissue surfaces in a patient. The macromer is mixed with a photoinitiator or photoinitiator/cocatalyst mixture to form an aqueous mixture and the mixture is applied to a tissue surface to which tissue adhesion is desired. The tissue surface is contacted with the tissue with which adhesion is desired, forming a tissue junction. The tissue junction is then irradiated until the macromers are polymerized.

5

Tissue Coatings

In a particularly preferred application of these macromers, an ultrathin coating is applied to the surface of a tissue, most preferably the lumen of a tissue such as a blood vessel. One use of such a coating is in the treatment or prevention of restenosis, abrupt reclosure, or vasospasm after vascular intervention. The photoinitiator is applied to the surface of the tissue, allowed to react, adsorb or bond to tissue, the unbound photoinitiator is removed by dilution or rinsing, and the macromer solution is applied and polymerized.

15 As demonstrated below, this method is capable of creating uniform polymeric coating of between one and 500 microns in thickness, most preferably about twenty microns, which does not evoke thrombosis or localized inflammation.

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20Tissue Supports

The macromers can also be used to create tissue supports by forming shaped articles within the body to serve a mechanical function. Such supports include, for example, sealants for bleeding organs, sealants for bone defects and space-filers for vascular aneurisms. Further, such supports 25 include strictures to hold organs, vessels or tubes in a particular position for a controlled period of time.

The invention described herein is further exemplified in the following Examples. While these Examples provide a

5 variety of combinations useful in performing the methods of the invention, they are illustrative only and are not to be viewed as limiting in any manner the scope of the invention.

Example 1

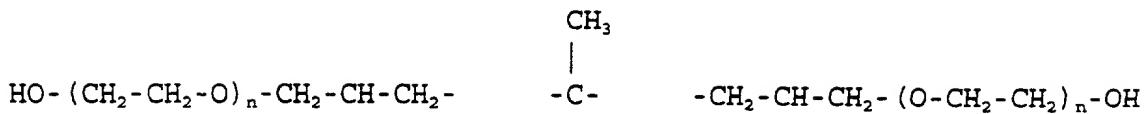
Synthesis of PEG 6 kD Diacrylate

10 PEG acrylates of molecular weights 400 Da and 1,000 Da
ware commercially available from Sartomer and Dajac Inc.,
respectively. PEG 6 kD (20 g) was dissolved in 200 mL
dichloromethane in a 250 mL round bottom flask. The flask was
cooled to 0°C and 1.44 mL of triethyl amine and 1.3 mL of
15 acryloyl chloride were added with constant stirring under a dry
nitrogen atmosphere. The reaction mixture was then brought to
room temperature and stirred for 12 hr under a nitrogen
atmosphere. It was then filtered, and the filtrate was
precipitated by adding to a large excess of hexane. The crude
20 monomer was purified by dissolving in dichloromethane and
precipitating in hexane. Yield 69%.

Example 2

Synthesis of PEG 18.4 kD Tetraacrylate

A tetrafunctional water soluble PEG (30 g; m.w. 18.5
25 kD) having the following structure was purchased from
Polysciences, Inc.:



5 where $F_1 = \text{CONH, COO or NHCOO}$

$X = \text{H, CH}_3, \text{C}_2\text{H}_5, \text{C}_6\text{H}_5, \text{Cl, Br, OH or CH}_2\text{COOH}$

$F_2 = \text{COO, CONH, O or C}_6\text{H}_4, \text{AND}$

$R = \text{CH}_2 \text{ or -alkyl-}.$

The PEG was dried by dissolving in benzene and
distilling off the water-benzene azeotrope. PEG 18.5 kD (59 g)
was dissolved in 300 mL of benzene in a 500 mL flask. To this,
3.6 mL of triethylamine and 2.2 mL of acryloyl chloride were
added under nitrogen atmosphere and the reaction mixture was
refluxed for 2 hours. It was then cooled and stirred overnight.
The triethyl amine hydrochloride was separated by filtration and
the copolymer was recovered from filtrate by precipitating in a
large excess of hexane. The polymer was further purified by
dissolving in methylene chloride and reprecipitating in hexane.
The polymer was dried at 50°C under vacuum for 1 day. Yield 68%.

20

Example 3

Coating of Islet-Containing Alginate-PLL

Microspheres by Surface Dye Adsorption

The microcapsule interfacial polymerization method was used to form membrane around alginate-PLL microcapsules

5 containing islets. Alginate-PLL coacervated microspheres,
containing one or two human pancreatic islets each, were
suspended in a 1.1% CaCl₂ solution and aspirated free of excess
solution to obtain a dense plug of microspheres. A solution of
ethyl eosin (0.04% w/v) was prepared in a 1.1 % CaCl₂ solution.
10 This solution was filter-sterilized by passage through a 0.45μm
filter. The plug of microspheres was suspended in 10 mL of the
eosin solution for 2 min to allow uptake of the dye. The
microspheres were then washed four times with fresh 1.1% CaCl₂ to
remove excess dye. A solution of PEG 18.5 tetraacrylate (2 mL;
15 23% w/v) containing 100μL of a 3.5% w/v solution of
triethanolamine in HEPES buffered saline was added to 0.5 mL of
these microspheres. The microspheres were exposed to argon ion
laser light for 30 seconds with periodic agitation. The
suspension of microspheres was uniformly scanned with the light
during this period. The microspheres were then washed with
calcium solution and the process was repeated in order to further
stabilize the coating.

A static glucose stimulation test (SGS) was performed
on islets encapsulated in the microspheres coated with PEG gel.
25 Data for insulin secretion in response to this challenge appears
in Table 1. The islets were seen to be viable by dithizone
staining. The SGS test data confirm the vitality and
functionality of the islets.

TABLE 1

SGS

		initial	pulse	subsequent
	<u>Glucose Concentration (mg%)</u>	60	300	60

Insulin/Islet/hr (μ U/mL)*

10	Diffusion Overcoat Method	1.0	10.04 \pm 3.56	2.5450.76
	Mineral Oil Overcoat Method	1.0	10.23 \pm 3.28	1.0250.78
	Free Islet Control	1.0	3.74 \pm 1.4	1.950.17

* Values are mean \pm S.D., all are normalized as compared to the initial 60 mg %, after subjection to the 300 mg % glucose, the islets were resubjected to the initial dose.

PEG diacrylate macromers may be polymerized identically as the PEG tetraacrylate macromer described in this example.

Example 4

Coating Islet-Containing Alginate-PLL Microspheres

20 by the Microcapsule Suspension Polymerization Method

This method takes advantage of the hydrophilic nature of PEG monomers. Alginate/PLL microspheres (2 mL), containing one or two human pancreatic islets each, were mixed with PEG tetraacrylate macromer solution (PEG mol wt 18.5 kD, 23% solution in saline) in a 50 mL transparent centrifuge tube.

25 Triethanolamine (0.1M) and 0.5 mM ethyl eosin were mixed with macromer solution. The excess of macromer solution was decanted,

5 20 mL of mineral oil was added to the tube, and the reaction mixture was vortexed thoroughly for 5 minutes. Silicone oil will perform equally well in this synthesis but may have poorer adjuvant characteristics if there is any carry-over. Any other water-immiscible liquid may be used as the "oil" phase.

10 Acceptable triethanolamine concentrations range from about 1 mM to about 100mM. Acceptable ethyl eosin concentrations range from about 0.01 mM to more than 10 mM.

15 The beads were slightly red due to the thin coating of macromer/dye solution, and they were irradiated for 20-50 sec with an argon ion laser (power 50-500 mW). Bleaching of the (red) ethyl eosin color suggested completion of the reaction. The beads were then separated from mineral oil and washed several times with saline solution. The entire procedure was carried out under sterile conditions.

20 A schematic representation of the microsphere coating process in oil is shown in Figure 3. Alginate/polylysine capsules are soluble in sodium citrate at pH 12. When these coated microspheres came in contact with sodium citrate at pH 12, the inner alginate/polylysine coacervate dissolves and a PEG 25 polymeric membrane could still be seen (crosslinked PEG gels are substantially insoluble in all solvents including water and sodium citrate at pH 12). The uncoated control microspheres dissolved completely and rapidly in the same solution.

5 A static glucose challenge was performed on the islets
as in Example 3. Data again appear in Table 1. The islets were
seen to be viable and functional.

Example 5

Encapsulation of Islets of Langerhans

10 This example makes use of the direct interfacial
polymerization. Islets of Langerhans isolated from a human
pancreas were encapsulated in PEG tetraacrylate macromer gels.
15 500 islets suspended in RPMI 1640 medium containing 10% fetal
bovine serum were pelleted by centrifuging at 100g for 3 min.
The pellet was resuspended in 1 mL of a 23% w/v solution of PEO
18.5 kD diacrylate macromer in HEPES buffered saline. An ethyl
eosin solution (5 μ L) in vinyl pyrrolidone (at a concentration of
0.5%) was added to this solution along with 100 μ L of a 5 M
solution of triethanolamine in saline. Mineral oil (20 mL) was
20 then added to the tube which was vigorously agitated to form a
dispersion of droplets 200-500 μ m in size. This dispersion was
then exposed to an argon ion laser with a power of 250 mW,
emitting at 514 nm, for 30 sec. The mineral oil was then
separated by allowing the microspheres to settle, and the
25 resulting microspheres were washed twice with PBS, once with
hexane and finally thrice with media.

Figure 4 shows islets of Langerhans encapsulated in a
PEO gel. The viability of the islets was verified by an acridine

5 orange and propidium iodide staining method and also by dithizone staining. In order to test functional normalcy, an SGS test was performed on these islets. The response of the encapsulated islets was compared to that of free islets maintained in culture for the same time period. All islets were maintained in culture 10 for a week before the SGS was performed. The results are summarized in Table 2. It can be seen that the encapsulated islets secreted significantly ($p<0.05$) higher insulin than the free islets. The PEO gel encapsulation process did not impair function of the islets and in fact helped them maintain their function in culture better than if they had not been 15 encapsulated.

TABLE 2

Islet Insulin secretion

<u>Glucose Concentration (mg%)</u>	60	300	60
Insulin/Islet/hr (μ U/mL)*			
Free islets	1.0	3.74+/-1.40	1.9+/-0.17
Encapsulated Islets	1.0	20.81+/-9.36	2.0+/-0.76

*Values are mean +/- S.D., normalized to initial basal level at

25 60 mg% glucose.

Example 6**Microencapsulation of Animal Cells**

PEG diacrylates of different molecular weight were synthesized by a reaction of acryloyl chloride with PEG as in Example 1. A 20 to 30% solution of macromer was mixed with a 10 cell suspension and the ethyl eosin and triethanolamine initiating system before exposing it to laser light through a coextrusion air flow apparatus, Figure 5. Microspheres were prepared by an air atomization process in which a stream of macromer was atomized by an annular stream of air. The air flow rate used was 1,600 cc/min and macromer flow-rate was 0.5 mL/min. The droplets were allowed to fall to a petri dish containing mineral oil and were exposed to laser light for about 0.15 sec each to cause polymerization and make them insoluble in water. Microspheres so formed were separated from the oil and thoroughly washed with PBS buffer to remove unreacted macromer and residual initiator. The size and shape of microspheres was dependent on extrusion rate (0.05 to 0.1 mL/min) and extruding capillary diameter (18 Ga to 25 Ga). The polymerization times were dependent on initiator concentration (ethyl eosin concentration 20 (5 μ M to 0.5mM), vinyl pyrrolidone concentration (0.0% to 0.1 %), triethanolamine concentration (5 to 100 mM), laser power (10mW to 25 1W), and macromer concentration (>10% w/v).

A PEG diacrylate macromer of molecular weight 400 Da was used as a 30% solution in PBS, containing 0.1M

5 triethanolamine as a cocatalyst and 0.5mM ethyl eosin as a photoinitiator. Spheres prepared using this method are shown in Figure 6. The polymerizations were carried out at physiological pH in the presence of air. This is significant since radical polymerizations may be affected by the presence of oxygen, and
10 the acrylate polymerization is still rapid enough to proceed effectively.

The process also works at lower temperatures. For cellular encapsulation, a 23% solution of PEO diacrylate was used with initiating and polymerization conditions as used in the air atomization technique. Cell viability subsequent to encapsulation was checked by trypan blue exclusion assay. Human foreskin fibroblasts (HFF), Chinese hamster ovary cells (CHO-K1), and a beta cell insuloma line (RiNSF) were found to be viable (more than 95%) after encapsulation. A wide range (> 10%) of PEG diacrylate concentrations may be used equally effectively, as may PEG tetraacrylate macromers.
15
20

Example 7

Coating of Animal Cell-Containing Alginate-PLL

Microspheres and Individual Cells by Surface Dye Adsorption

25 Alginate-PLL coacervated microspheres, containing animal cells, were suspended in a 1.1% CaCl₂ solution and were aspirated free of excess solution to obtain a dense plug of microspheres. A solution was filter sterilized by passage

5 through a 0.45 pm filter. The plug of microspheres was suspended
in 10 mL of eosin solution for 2 min to allow dye uptake. A
solution of PEG 18.5 tetraacrylate (2 mL; 23% w/v) containing 100
 μ L of a 3.5 w/v solution of triethanolamine in HEPES buffered
saline was added to 0.5 mL of these microspheres. The
10 microspheres were exposed to an argon ion laser for 30 seconds
with periodic agitation. The suspension of microspheres was
uniformly scanned with the laser during this period. The
microspheres were then washed with calcium solution and the
process was repeated once more in order to attain a stable
coating.

15 In order to verify survival of cells after the overcoat
process, cells in suspension without the alginate/PLL micro-
capsule were exposed to similar polymerization conditions. 1 mL
of lymphoblastic leukemia cells (RAJI) (5×10^5 cells) was centri-
fuged at 300 g for 3 min. A 0.04% filter sterilized ethyl eosin
solution in phosphate buffered saline (PBS) (1 mL) was added and
the pellet was resuspended. The cells were exposed to the dye
for 1 min and washed twice with PBS and then pelleted.
20 Triethanolamine solution (10 μ L; 0.1M) was added to the pellet
and the tube was vortexed to resuspend the cells. 0.5 mL of PEO
18.5 kD tetraacrylate macromer was then mixed along with this
suspension and the resulting mixture was exposed to an argon ion
laser (514 nm, 50 mW) for 45 sec. The cells were then washed
twice with 10 mL saline and once with media (RPMI 1640 with 10%

5 FCS and 1% antibiotic, antimycotic). A thin membrane of PEO gel
may be observed forming around each individual cell.

No significant difference in viability was seen between
the control population (93% viable) and the treated cells (95%
viable) by trypan blue exclusion. An assay for cell viability
10 and function was performed by adapting the MTT-Formazan assay for
the RAJI cells. This assay indicates > 90% survival. Similar
assays were performed with two other model cell lines. Chinese
hamster ovary cells (CHO-K1) show no significant difference
(p<0.05) in metabolic function as evaluated by the MTT-Formazan
assay. 3T3 mouse fibroblasts also show no significant reduction
(p<0.05) in metabolic activity.

Example 8

Coating Animal Cell Containing Alginate-PLL

Microspheres by the Oil Suspension Method

Using the method described in Example 4, RAJI cells
contained in alginate-PLL microspheres were coated with a PEG
polymeric membrane. Viability of these cells was checked by
trypan blue exclusion and they were found to be more than 95%
viable.

5

Example 9**Coating of Individual Islets of
Langerhans by Surface Dye Adsorption**

Using the method described in Example 7, ethyl eosin was adsorbed to the surfaces of islets, exposed to a solution of 10 the PEG macromer with triethanolamine, and exposed to light from an argon-ion laser to form a thin PEG polymeric membrane on the surface of the islets. Islet viability was demonstrated by lack of staining with propidium iodide.

Example 10**Biocompatibility of PEO on Microspheres**

In vivo evaluation of the extent of inflammatory response to microspheres prepared in Examples 7 and 8 was carried out by implantation in the peritoneal cavity of mice. Approximately 0.5 mL of microspheres were suspended in 5 mL of 15 sterile HEPES buffered saline. A portion of this suspension (2.5 mL) was injected into the peritoneal cavity of ICR male Swiss white mice. The microspheres were recovered after 4 days by conducting a lavage of the peritoneal cavity with 5 mL of 10U heparin/mL PBS. The extent of cellular growth on the 20 microspheres was visually inspected under a phase contrast microscope. The number of unattached cells present in the recovered lavage fluid was counted using a Coulter counter.

Figure 7A shows a photograph of alginate-poly(L-lysine)

5 microspheres explanted after 4 days, while Figure 7B shows
similar spheres which had been coated with PEG gel by the dye
diffusion process before implantation. As expected, bilayer
10 alginate-polylysine capsules not containing an outer alginate
layer to provide an extreme test of the ability of the PEG gel
layer to enhance the biocompatibility of the bilayer membrane,
were completely covered with cells due to the highly cell
adhesive nature of the PLL surface, whereas the PEG coated
microspheres were virtually free of adherent cells. Almost
15 complete coverage of alginate-poly(L-lysine) was expected because
polylysine has amino groups on the surface, and positively
charged surface amines can interact with cell surface
proteoglycans and support cell growth (Reuveny, et al., (1983)
Biotechnol. Bioeng., 25:469-480). The photographs in Figure 7B.
strongly indicate that the highly charged and cell adhesive
20 surface of PLL is covered by a stable layer of PEG gel. The
integrity of the gel did not appear to be compromised.

The non-cell-adhesive tendency of these microspheres
was evaluated as a percentage of the total microsphere area which
appears covered with cellular overgrowth. These results are
25 summarized in Table 3.

TABLE 3

Microsphere Coverage with Cell Overgrowth

Composition of PEG gel	% Cell coverage
18.5 kD	<1
18.5 kD 90%:0.4 kD 10%	<1
18.5 kD 50%:0.4 kD 50%	<1
/ 35k 90%:0.4 kD 10%	5-7
35k 50%: 0.4 kD 50%	<1
Alginic poly(L-lysine)	60-80

An increase in cell count was a result of activation of resident macrophages which secrete chemical factors such as interleukins and induce nonresident macrophages to migrate to the implant site. The factors also attract fibroblasts responsible for collagen synthesis. The variation of cell counts with chemical composition of the overcoat is shown Figure 8 (A-F). It can be seen from the figure that all PEG coated spheres have substantially reduced cell counts. This is consistent with the PEG overcoat generally causing no irritation of the peritoneal cavity.

However, PEG composition does make a difference in biocompatibility, and increasing molecular weights were associated with a reduction in cell counts. This could be due to the gels made from higher molecular weight oligomers having higher potential for steric repulsion due to the longer chain lengths.

5

Example 11**Permeability of PEO Gels**

Bovine serum albumin, human IgG, or human fibrinogen (20 mg) was dissolved in 2 mL of a 23% w/v solution of oligomeric PEO 18.5 kD tetraacrylate in PBS. This solution was laser polymerized to produce a gel 2cm X 2cm X 0.5 cm in size. The diffusion of bovine serum albumin, human IgG and human fibrinogen (mol wt 66 kD, 150 kD and 350 kD respectively) was monitored through the 2cm X 2cm face of these gels using a total protein assay reagent (Biorad). A typical release profile for a PEO 18.5 kD gel is shown in Figure 9. This gel allowed a slow transport of albumin but did not allow IgG and fibrinogen to diffuse. This indicates that these gels are capable of being used as immunoprotective barriers. This is a vital requirement for a successful animal tissue microencapsulation material.

The release profile was found to be a function of crosslink density and molecular weight of the polyethylene glycol segment of the monomer. Figure 10 shows the release of BSA through gels made from 23% solutions of PEO diacrylates and tetraacrylates of 0.4 kD and 18.5 kD, respectively. It is evident that the 18.5 kD gel is freely permeable to albumin while the 0.4 kD gel restricted the diffusion of albumin. The release of any substance from these gels will depend on the crosslink density of the network and will also depend on the motility of

5 the PEG segments in the network. This effect is also dependent upon the functionality of the macromer. For example, the permeability of a PEG 18.5 kD tetraacrylate gel is less than that of an otherwise similar PEG 20 kD diacrylate gel.

In the case of short PEO chains between crosslinks, the
10 "pore" produced in the network will have relatively rigid boundaries and will be relatively small and so a macromolecule attempting to diffuse through this gel will be predominantly restricted by a sieving effect. If the chain length between crosslinks is long, the chain can fold and move around with a
15 high motility and, besides the sieving effect, a diffusing macromolecule will also encounter a free volume exclusion effect.

Due to these two contrasting effects a straightforward relation between molecular weight cutoff for diffusion and the molecular weight of the starting oligomer is not completely definable. Yet, a desired release profile for a particular protein or a drug such as a peptide may be accomplished by adjusting the crosslink density and length of PEG segments.
Correspondingly, a desired protein permeability profile may be arranged to permit the diffusion of nutrients, oxygen, carbon
20 dioxide, waste products, hormones, growth factors, transport proteins, and released cellularly synthesized proteins, while restricting the diffusion of antibodies and complement proteins and also the ingress of cells, to provide immunoprotectivity to transplanted cells or tissue. The three dimensional crosslinked

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5 covalently bonded polymeric network is chemically stable for long-term in vivo applications.

Example 12

Treatment of Silicone Rubber to Enhance Biocompatibility

Pieces of medical grade silicone rubber (2 X 2 cm) were 10 soaked for 1 h in benzene containing 23% 0.4 kD PEG diacrylate and 0.5% 2,2-dimethoxy-2-phenyl acetophenone. The thus swollen rubber was irradiated for 15 min with a long wave UV lamp (365 nm). After irradiation, the sample was rinsed in benzene and dried. The air contact angles of silicone rubber under water 15 were measured before and after treatment. The decreased contact angle of 50° after treatment, over the initial contact angle of 63° for untreated silicone rubber reflects an increased hydrophilicity due to the presence of the PEG gel on the rubber surface.

This technique demonstrates that macromer 20 polymerization can be used to modify a polymer surface so as to enhance biocompatibility. For instance, a polyurethane catheter can be treated by this method to obtain an implantable device coated with PEG. The PEG was firmly anchored to the surface of 25 the polyurethane catheter because the macromer was allowed to penetrate the catheter surface (to a depth of 1-2 microns) during the soaking period before photopolymerization. Upon irradiation,

5 an interpenetrating network of PEG and polyurethane results. The
PEG was thereby inextricably intertwined with the polyurethane.

Example 13

Treatment of Polyurethane

INTRACATH (Becton Dickinson) polyurethane intravenous
10 catheters (19 ga) were modified at their outer surfaces with
polyethylene glycol diacrylate (PEG DA) of molecular weight 400
and 10000. The prepolymer was dissolved in tetrahydrofuran
(THF), a solvent for the polyurethane, at 50°C, where
polyurethane dissolution is relatively slow. The following
15 solution was prepared and warmed to 50°C:

PEG DA (MW 400)	15%
PEG DA (MW 10000)	15%
THF	70%

20 with 2,2-dimethoxy, 2-phenyl actophenone at 1.6% of the above
solution.

2.5" length catheter segments were closed at one end by
melting a 2 mm length by pressing with a hot metal spatula to
from a flat tab. This tab was used to fix the catheter in the
vessel wall in subsequent animal experiments. The catheter was
25 held with forceps at the tab end and dipped in the treatment
solution for 1-3 sec, pulled out, and the excess fluid shaken
off. The treated catheter was illuminated with an ultraviolet
light (Black Ray, 360 nm) for 2-3 min, rotating the catheter. An

5 untreated control was similarly treated in 70% THF with 30% water replacing the PEG in the treatment solution.

Following this treatment, both the treated and control catheters were transferred to 100% methylene chloride to extract unreacted materials; this extraction was carried out for 36 hr
10 with solvent replacement every 6 hr. These catheters were then dried and transferred to 70% ethanol, and then into water before use.

A second composition was also investigated:

PEG DA (MW 400)	10%
PEG DA (MW 10000)	15%
Polyethylene oxide (MW 100,000)	5%
THF	70%

with 2,2-dimethoxy, 2-phenyl acetophenone at 1.6% of the above solution.

In this case, the polyethylene oxide of mw 100,000 was not a prepolymer and was immobilized within the PEG DA matrix by entanglement, rather than by chemical attachment.

Adult New Zealand male rabbits (7-10 lb) were anesthetized with rompun-acepromazien-ketamine. The animal was
25 shaved on the ventrolateral jugular and the vessel was raised. A catheter was inserted into the vessel with the tab outside, and tied in place via the tab with 4.0 nylon to the adventitia. The catheter was inserted 1.5 to 2.0" into the vessel. The skin incision was closed.

5 After a period of 3 days, the animals were euthanized by overdose of pentobarbital intraperitoneally. The vessel was again raised and flushed with phosphate buffered saline (PBS) to superficially rinse away blood between the catheter and the vessel wall. Two 500 ml bottles, one filled with PBS and one
10 with formalin in PBS were hung from an i.v. pole scaffold, and the hydraulic differential was used to perfusion fix the vessel. The vessels were removed proximal and distal to the ends of the catheters.

15 The treated catheters were completely wettable, and were very slippery.

20 A total of 12 rabbits were catheterized for 72 hr. Six were control, unmodified catheters. These catheters could not be removed from the vessel wall without dissection, i.e. they were tightly incorporated into the vessel. These catheters upon removal were red, and the vessel was barely patent. By contrast, the treated catheters were easily removable, the vessels were clearly patent, and the catheters were not red. Under the light microscope, a small amount of white thrombus could be seen on both formulations of the catheter coating, with somewhat lesser amounts on the formulation containing the polyethylene oxide
25 100,000.

Example 14**Treatment of Ultrafiltration Membranes**

The processes of Examples described above can be applied to the treatment of macrocapsular surfaces, such as those used for ultrafiltration, hemodialysis and non-microencapsulated immunoisolation of animal tissue. The macrocapsule in this case will usually be microporous with a molecular weight cutoff below 70,000 Da. It may be in the form of a hollow fiber, a spiral module, a flat sheet or other configuration. The surface of such a macrocapsule can easily be modified using the PEO gel coating process to produce a non-fouling, non-thrombogenic, and non-cell-adhesive surface. The coating serves to enhance biocompatibility and to offer additional immunoprotection. Materials which can be modified in this manner include polysulfones, cellulosic membranes, polycarbonates, polyamides, polyimides, polybenzimidazoles, nylons, and poly(acrylonitrile-co-vinyl chloride) copolymers and the like.

Depending on the physical and chemical nature of the surface a variety of methods can be employed to form biocompatible overcoats. Hydrophilic surfaces can simply be coated by applying a thin layer of a 30% w/v polymerizable solution of PEG diacrylate containing appropriate amounts of dye and amine. Hydrophobic surfaces can be first rendered hydrophilic by gas plasma discharge treatment and the resulting surface can then be similarly coated, or they may simply be

5 treated with a surfactant before or during treatment with the PEG diacrylate solution.

Example 15

Treatment of Textured Materials and Hydrogels

The surface of materials having a certain degree of
10 surface texture, such as woven dacron, dacron velour, and expanded poly(tetrafluoroethylene) (ePTFE) membranes, was treated using the coating method described herein. Textured and macroporous surfaces allow greater adhesion of the PEG gel to the material surface. This allows the coating of relatively
15 hydrophobic materials such as PTFE and poly(ethylene terephthalate) (PET).

Implantable materials such as enzymatic and ion sensitive electrodes, having a hydrogel (such as poly (HEMA), crosslinked poly(vinyl alcohol) and poly(vinyl pyrrolidone)) on their surface, are coated with the more biocompatible PEO gel in a manner similar to the dye adsorption and polymerization technique used for the alginate-PLL microspheres.

Example 16

Treatment of Dense Materials

25 The surfaces of dense (e.g., nontextured, nongel) materials such as polymers (including PET, PTFE, polycarbonates, polyamides, polysulfones, polyurethanes, polyethylene,

5 polypropylene, polystyrene), glass, and ceramics can be treated
with PEO gel coatings. Hydrophobic surfaces were initially
treated by a gas plasma discharge to render the surface
hydrophilic. This ensures better adhesion of the PEO gel coating
to the surface. Alternatively, coupling agents may be used to
10 increase adhesion, as readily apparent to those skilled in the
art of polymer synthesis.

Example 17

Rate of Polymerization

To demonstrate rapidity of gelation in laser-initiated
polymerizations of multifunctional acrylic monomers, the kinetics
of a typical reaction were investigated. Trimethylolpropyl tri-
acrylate containing 5×10^{-4} M ethyl eosin as a photoinitiator in
10 μ moles of N-vinyl pyrrolidone per mL of macromer mix and 0.1M
of triethanolamine as a cocatalyst, was irradiated with a 500 mW
20 argon ion laser (514 nm wavelength, power 3.05×10^5 W/m², beam
diameter 1 mm, average gel diameter produced 1 mm). A plot of
the length of the spike of gel formed by penetration of the laser
beam into the gel versus laser irradiation time is shown in
Figure 11A. The spikes formed as a result of laser light
25 penetration into the macromer can be seen in Figure 11B.

A 23% w/w solution of various macromers in HEPES
buffered saline containing 3 μ L of initiator solution (300 mg/mL
of 2,2-dimethoxy-2-phenylacetophenone in N-vinyl pyrrolidone) was

5 used. 100 μL of the solution was placed on a glass coverslip and
irradiated with a low intensity long wave UV (LWUV) lamp
(BlakRay, model 3-100A with flood). The times required for
gelation to occur were noted and are given in Table 4. These
times were typically in the range of 10 seconds.

10

TABLE 4

Gelling Time

Polymer Code	Gel Time (sec) (mean \pm S.D.)
0.4 kD	6.9 \pm 0.5
1 kD	21.3 \pm 2.4
6 kD	14.2 \pm 0.5
10 kD	8.3 \pm 0.2
18.5 kD	6.9 \pm 0.1
20 kD	9.0 \pm 0.4

15
0
G
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Time periods of about 10-100 ms were sufficient to gel
a 300 μm diameter droplet (a typical size of gel used in
microencapsulation technology). This rapid gelation, if used in
conjunction with proper choice of macromers, can lead to
entrapment of living cells in a three dimensional covalently
25 bonded polymeric network. The monochromatic laser light will not
be absorbed by the cells unless a proper chromophore is present,
and is considered to be harmless if wavelength is more than about
400 nm. Exposure to long wavelength ultraviolet light (>360 nm)
is harmless at practical intensities and durations.

5

Example 18

PEO Gel Interactions

Biocompatibility with HFF (human foreskin fibroblasts) cells was demonstrated as follows.

HFF cells were seeded on PEO 18.5 kD tetraacrylate gels at a density of 18,000 cells/cm² in Dulbecco's modification of Eagle's medium containing 10% fetal calf serum. The gels were then incubated at 37°C in a 5% CO₂ environment for 4 hr. At the end of this time the gels were washed with PBS to remove any non-adherent cells and were observed under a phase contrast microscope at a magnification of 200X. Figure 12A shows the growth of these cells on a typical PEG gel as compared to glass surface (Figure 12B). The number of attached cells/cm² was found to be 510 ± 170 on the gel surfaces as compared to 13,200 ± 3,910 for a control glass surface. The cells on these gels appeared rounded and were not in their normal spread morphology, strongly indicating that these gels do not encourage cell attachment.

Biocompatibility on microspheres was demonstrated as follows. Figure 13 shows a photograph of microspheres explanted from mice as in Example 10; after 4 days very little fibrous overgrowth was seen. The resistance of PEG chains to protein adsorption and hence cellular growth was well documented. Table 5 summarizes the extent of cellular overgrowth seen on these microspheres after 4 day intraperitoneal implants for various PEG diacrylate gels.

TABLE 5

PEG Diacrylate for Gels Extent of Cellular Overgrowth
(mol wt, Daltons)

	400	5-10%
10	1,000	15-25%
	5,000	3-5%
	6,000	2-15%
	10,000	10-20%
	18,500	4-10%

Example 19

Characterization and Mechanical Analysis of PEO Gels

Solutions of PEO diacrylates (23% w/v; 0.4 kD, 6 kD, 10 kD) and PEG tetraacrylates (18.5 kD) were used. An initiator solution (10 μ L) containing 30 mg/mL of 2,2-dimethoxy-2-phenyl acetophenone in vinyl-2-pyrrolidone was used per mL of the macromer solution. The solution of initiator containing macromer was placed in a 4.0 X 1.0 X 0.5 cm mold and exposed to a long wave ultraviolet lamp (365 nm) for approximately 10 seconds to induce gelation. Samples were allowed to equilibrate in phosphate buffered saline (pH 7.4) for 1 week before analysis 1 performed.

5 A series of "dogbone" samples (samples cut from a slab
into the shape of a dogbone, with wide regions at both ends and a
narrower long region in the middle) were cut for ultimate tensile
strength tests. Thickness of the samples was defined by the
thickness of the sample from which they were cut. These thick-
nesses ranged from approximately 0.5 mm to 1.75 mm. The samples
were 20 mm long and 2 mm wide at a narrow "neck" region. The
stress strain tests were run in length control at a rate of 4%
per second. After each test, the cross sectional area was
determined. Table 6 shows the ultimate tensile strength data.
515 It is seen that the lower molecular weight macromers in general
give stronger gels which were less extensible than those made
using the higher molecular weight macromers. The PEG 18.5 kD
tetraacrylate gel is seen to be anomalous in this series,
resulting from the multifunctionality of the macromer and the
corresponding higher crosslinking density in the resulting gel.
20 This type of strengthening result could be similarly achieved
with macromers obtained having other than four free radical
sensitive groups, such as acrylate groups.

TABLE 6

Gel strength Tests

	PEO Acrylate Precursor Molecular Weight			
	0.4 kD	6 kD	10 kD	18.5 kD
10				
Stress (kPa)*	168+/-51	98+/-15	33+/-7	115+/-56
% Strain*	8+/-3	71+/-13	110+/-9	40+/-15
Slope*	22+/-5	1.32+/-0.31	0.27+/-0.04	2.67+/-0.55

15 *Values are mean+/-S.D.

For the creep tests, eight samples approximately 0.2 X 0.4 X 2 cm were loaded while submersed in saline solution. They were tested with a constant unique predetermined load for one hour and a small recovery load for ten minutes. Gels made from PEG diacrylates of 1 kD, 6 kD, and 10 kD, and PEG tetraacrylates of 18.5 kD PEO molecular weight were used for this study. The 10 kD test was terminated due to a limit error (the sample stretched beyond the travel of the loading frame). The 1 kD sample was tested with a load of 10g and a recovery load of 0.2g. The 6 kD sample was tested at a load of 13g with a recovery load of 0.5g. The 18.5 kD sample was tested at a load of 13g with a recovery load of 0.2g. The choice of loads for these samples produced classical creep curves with primary and secondary regions. The

5 traces for creep for the 1 kD, 6 kD, and 18.5 kD samples appear
in Figure 14A-C, respectively.

Example 20

Water Content of PEO Gels

Solutions of various macromers were made as described
10 above. Gels in the shape of discs were made using a mold. The
solutions (400 μ L) was used for each disc. The solutions were
irradiated for 2 minutes to ensure thorough gelation. The disc
shaped gels were removed and dried under vacuum at 60°C for 2
days. The discs were weighed (W1) and then extracted repeatedly
15 with chloroform for 1 day. The discs were dried again and
weighed (W2). The gel fraction was calculated as W2/W1. This
data appears in Table 7.

Determination of Degree of Hydration

Subsequent to extraction, the discs were allowed to
20 equilibrate with HBS for 6 hours and weighed (W3) after excess
water had been carefully swabbed away. The total water content
was calculated as (W3-W2) X 100/W3. The data for gel water
contents is summarized in the following table.

5

TABLE 7

Polymer Coat	% Total Water	% Gel Content
0.4 kD	-	99.8 ± 1.9
1 kD	79.8 ± 2.1	94.5 ± 2.0
6 kD	95.2 ± 2.5	69.4 ± 0.6
10 kD	91.4 ± 1.6	96.9 ± 1.5
18.5 kD	91.4 ± 0.9	80.3 ± 0.9
20 kD	94.4 ± 0.6	85.0 ± 0.4

Example 21**Mechanical Stability of PEO Gels after Implantation**

PEG diacrylate (10 kD) and PEG tetraacrylate (18.5 kD) were cast in dogbone shapes as described in Example 19. PEG-diacrylate or tetraacrylate (23% w/w) in sterile HEPES buffered saline (HBS) (0.9% NaCl, 10 - HEPES, pH 7.4) containing 900 ppm of 2,2-dimethoxy-2-phenoxyacetophenone as initiator, was poured into an aluminum mold and irradiated with a LWUV lamp (Black ray) for 1 min. The initial weights of these samples were found after oven-drying these gels to constant weight. The samples were soxhlet-extracted with methylene chloride for 36 hours in order to leach out any unreacted prepolymer from the gel matrix (sol-leaching) prior to testing. The process of extraction was continued until the dried gels gave constant weight.

ICR Swiss male white mice, 6-8 weeks old (Sprague-Dawley), were anesthetized by an intraperitoneal injection of

5 sodium pentobarbital." The abdominal region of the mouse was shaved and prepared with betadine. A ventral midline incision 10-15 mm long was made. The polymer sample, fully hydrated in sterile PBS (Phosphate buffered saline) or HEPES buffered saline (for calcification studies), was inserted through the incision 10 and placed over the mesentery, away from the wound site. The peritoneal wall was closed with a lock stitched running suture (4.0 silk, Ethicon). The skin was closed with stainless steel skin staples, and a topical antibiotic (Furacin) was applied over the incision site. Three animals were used for each time point. 15 One dogbone sample was implanted per mouse and explanted at the end of 1 week, 3 weeks, 6 weeks, and 8 weeks. Explanted gels were rinsed in HBS twice and then treated with 0.3 mg/mL pronase (Calbiochem) to remove any adherent cells and tissue. The samples were then oven-dried to a constant weight, extracted, and reswelled as mentioned before.

20 Tensile stress strain test was conducted on both control (unimplanted) and explanted dogbones in a small horizontal Instron-like device. The device is an aluminum platform consisting of two clamps mounted flat on a wooden board between two parallel aluminum guide. The top clamp was 25 stationary while the bottom clamp was movable. Both the frictional surfaces of the moving clamp and the platform were coated with aluminum backed Teflon (Cole-Parmer) to minimize frictional resistance. The moving clamp was fastened to a device

5 capable of applying a gradually increasing load. The whole set up
was placed horizontally under a dissecting microscope (Reichert)
and the sample elongation was monitored using a video camera.
The image from the camera was acquired by an image processor
(Argus-10, Hamamatsu) and sent to a monitor. After breakage, a
10 cross section of the break surface was cut and the area measured.
The load at break was divided by this cross section to find the
maximum tensile stress. Table 8 lists the stress at fracture of
PEG tetraacrylate (18.5 kD) hydrogels explanted at various time
intervals. No significant change in tensile strength was evident
15 with time. Thus, the gels appear mechanically stable to
biodegradation in vivo within the maximum time frame of implant
in mice.

TABLE 8

TIME	STRESS (KPa)	STRAIN AV.
	IMPLANTED	(mean ± error*)
1 WK	52.8 ± 16.7	0.32 ± 0.19
3 WK	36.7 ± 10.6	0.37 ± 0.17
6 WK	73.3 ± 34.9	0.42 ± 0.26
8 WK	34.1†	0.30†
25 CONTROL	44.9 ± 5.3	0.22 ± 0.22

* Error based on 90% confidence limits.

† Single sample.

5

Example 22**Monitoring of Calcification of PEO Gels**

Disc shaped PEG-tetraacrylate hydrogels (m.w. 18.5 kD) were implanted intraperitoneally in mice as mentioned above for a period of 1 week, 3 weeks, 6 weeks, or 8 weeks. Explanted gels 10 were rinsed in HBS twice and treated with Pronase (Calbiochem) to remove cells and cell debris. The samples were then equilibrated in HBS to let free Ca⁺⁺ diffuse out from the gel matrix. The gels were then oven-dried (Blue-M) to a constant weight and transferred to Aluminum oxide crucibles (COORS, high temperature 15 resistant). They were incinerated in a furnace at 700°C for at least 16 hours. Crucibles were checked for total incineration, if any residual remnants or debris was seen they were additionally incinerated for 12 hours. Subsequently, the crucibles were filled with 2 mL of 0.5 M HCl to dissolve Ca⁺⁺ salt and other 20 minerals in the sample. This solution was filtered and analyzed with atomic absorption spectroscopy (AA) for calcium content.

25

Calcification data on PEG-tetraacrylate (mol. wt. 18.5 kD) gel implants is given in Table 9. No significant increase in calcification was observed up to an 8 week period of implantation in mice.

TABLE 9

	TIME (Days)	CALCIFICATION (mean \pm error*) (mg Calcium/g of Dry gel wt.)
	7	2.33 \pm 0.20
	21	0.88 \pm 0.009
10	42	1.08 \pm 0.30
	56	1.17 \pm 0.26

* Error based on 90% confidence limits.

Example 23

Encapsulation of Neurotransmitter-Releasing Cells

Paralysis agitans, more commonly called Parkinson's disease, is characterized by a lack of the neurotransmitter dopamine within the striatum of the brain. Dopamine secreting cells such as cells from the ventral mesencephalon, from neuroblastoid cell lines or from the adrenal medulla can be encapsulated in a manner similar to that of other cells mentioned in prior Examples. Cells (including genetically engineered cells) secreting a precursor for a neurotransmitter, an agonist, a derivative or a mimic of a particular neurotransmitter or analogs can also be encapsulated.

Example 24**Encapsulation of Hemoglobin for Synthetic Erythrocytes**

Hemoglobin in its free form can be encapsulated in PEG gels and retained by selection of a PEG chain length and cross-link density which prevents diffusion. The diffusion of hemoglobin from the gels may be further impeded by the use of polyhemoglobin, which is a cross-linked form of hemoglobin. The polyhemoglobin molecule is too large to diffuse from the PEG gel. Suitable encapsulation of either native or crosslinked hemoglobin may be used to manufacture synthetic erythrocytes. The entrapment of hemoglobin in small spheres (< 5 μ m) of these highly biocompatible materials would lead to enhanced circulation times relative to crosslinked hemoglobin or liposome encapsulated hemoglobin.

Hemoglobin in PBS is mixed with the prepolymer in the following formulation:

Hemoglobin at the desired amount

PEG DA (MW 10000) 35%

PEG DA (MW 1000) 5%

PBS 60%

with 2,2-dimethoxy, 2-phenyl acetophenone at 1.6% of the above solution.

This solution is placed in mineral oil at a ratio of 1 part hemoglobin/prepolymer solution to 5 parts mineral oil and is rapidly agitated with a motorized mixer to form an emulsion.

5 This emulsion is illuminated with a long-wavelength ultraviolet light (360nm) for 5 min to crosslink the PEG prepolymer to form a gel. The mw of the prepolymer may be selected to resist the diffusion of the hemoglobin from the gel, with smaller PEG DA molecular weights giving less diffusion. PEG DA of MW 10000,
10 further crosslinked with PEG DA 1000, should possess the appropriate permselectivity to restrict hemoglobin diffusion, and it should possess the appropriate biocompatibility to circulate within the bloodstream.

Example 25

**Entrapment of Enzymes for Correction of
Metabolic Disorders and Chemotherapy**

Congenital deficiency of the enzyme catalase causes acatalasemia. Immobilization of catalase in PEG gel networks could provide a method of enzyme replacement to treat this disease. Entrapment of glucosidase can similarly be useful in treating Gaucher's disease. Microspherical PEG gels entrapping urease can be used in extracorporeal blood to convert urea into ammonia. Enzymes such as asparaginase can degrade amino acids needed by tumor cells. Immunogenicity of these enzymes prevents direct use for chemotherapy. Entrapment of such enzymes in immunoprotective PEG gels, however, can support successful chemotherapy. A suitable formulation can be developed for either slow release or no release of the enzyme.

5 Catalase in PBS is mixed with the prepolymer in the
following formulation:

Catalase at the desired amount

PEG DA (MW 10000) 35%

PEG DA (MW 1000) 5%

10 PBS 60%

with 2,2-dimethoxy, 2-phenyl acetophenone at 1.6% of the above
solution.

This solution is placed in mineral oil at a ratio of 1
part catalase/prepolymer solution to 5 parts mineral oil and is
15 rapidly agitated with a motorized mixer to form an emulsion.
This emulsion is illuminated with a long-wavelength ultraviolet
light (360nm) for 5 min to crosslink the PEG prepolymer to form a
gel. The mw of the prepolymer may be selected to resist the
diffusion of the catalase from the gel, with smaller PEG DA
20 molecular weights giving less diffusion.

PEG DA of MW 10,000, further crosslinked with PEG DA
1000, should possess the appropriate permselectivity to restrict
catalase diffusion, and it should possess the appropriate
permselectivity to permit the diffusion of hydrogen peroxide into
25 the gel-entrapped catalase to allow the enzymatic removal of the
hydrogen peroxide from the bloodstream. Furthermore, it should
possess the appropriate biocompatibility to circulate within the
bloodstream.

5 In this way, the gel is used for the controlled
containment of a bioactive agent within the body. The active
agent (enzyme) is large and is retained within the gel, and the
agent upon which it acts (substrate) is small and can diffuse
into the enzyme rich compartment. However, the active agent is
10 prohibited from leaving the body or targeted body compartment
because it cannot diffuse out of the gel compartment.

Example 26

Use of PEO Gels as Adhesive to Rejoin Severed Nerve

15 A formulation of PEG tetraacrylate (10%, 18.5K), was
used as adhesive for stabilizing the sutureless apposition of the
ends of transected sciatic nerves in the rat. Rats were under
pentobarbital anesthesia during sterile surgical procedures. The
sciatic nerve was exposed through a lateral approach by
20 deflecting the heads of the biceps femoralis at the mid-thigh
level. The sciatic nerve was mobilized for approximately 1 cm
and transected with iridectomy scissors approximately 3 mm
proximal to the tibial-peroneal bifurcation. The gap between the
ends of the severed nerves was 2-3 mm. The wound was irrigated
25 with saline and lightly swabbed to remove excess saline.

Sterile, unpolymerized PEG tetraacrylate solution was applied to
the wound. Using delicate forceps to hold the adventitia or
perineurium, the nerve ends were brought into apposition, the

5 macromer solution containing 2,2-dimethoxy-2-phenoxyacetophenone as a photoinitiator applied to the nerve ends and the wound was exposed to long wavelength UV-light (365 nm) for about 10 sec to polymerize the adhesive. The forceps were gently pulled away. Care was taken to prevent the macromer solution from flowing 10 between the two nerve stumps. Alternatively, the nerve stump junction was shielded from illumination, e.g., with a metal foil, to prevent gelation of the macromer solution between the stumps; the remaining macromer solution was then simply washed away.

In an alternative approach, both ends of the transected nerve can be held together with one pair of forceps. Forceps 15 tips are coated lightly with petrolatum to prevent reaction with the adhesive.

The polymerized adhesive serves to encapsulate the wound and adhere the nerve to the underlying muscle. The 20 anastomosis of the nerve ends resists gentle mobilization of the joint, demonstrating a moderate degree of stabilization. The muscle and skin were closed with sutures. Re-examination after one month shows that severed nerves remain reconnected, despite unrestrained activity of the animals.

Example 27**Surgical Adhesive**

Abdominal muscle flaps from female New Zealand white rabbits were excised and cut into strips 1 cm X 5 cm. The flaps were approximately 0.5 to 0.8 cm thick. The lap joint, 1 cm X 1 cm, was made using two such flaps. Two different PEO di- and tetra-acrylate macromer compositions, 0.4K (di-) and 18.5K (tetra-), were evaluated. The 0.4K composition was a viscous liquid and was used without further dilution. The 18.5K composition was used as a 23% w/w solution in HBS. 125 μ l of ethyl eosin solution in n-vinyl pyrrolidone (20 mg/ml) along with 50 μ l of triethanolamine was added to each ml of the adhesive solution. 100 μ l of adhesive solution was applied to each of the overlapping flaps. The lap joint was then irradiated by scanning with a 2 W argon ion laser for 30 seconds from each side. The strength of the resulting joints was evaluated by measuring the force required to shear the lap joint. One end of the lap joint was clamped and an increasing load was applied to the other end, while holding the joint horizontally until it failed. Four joints were tested for each composition. The 0.4K joints had a strength of 12.0 ± 6.9 KPa (mean \pm S.D.), while the 18.5K joints had a strength of 2.7 ± 0.5 KPa. It is significant to note that it was possible to achieve photopolymerization and reasonable joint strength despite the 6-8 mm thickness of tissue. A

5 spectrophotometric estimate using 514 nm light showed less than
1% transmission through such muscle tissue.

Example 28

Modification of polyvinyl alcohol

2 g of polyvinyl alcohol (mol wt 100,000-110,000) was
10 dissolved in 20 ml of hot DMSO. The solution was cooled to room
temperature and 0.2 ml of triethylamine and 0.2 ml of acryloyl
chloride was added with vigorous stirring, under an argon
atmosphere. The reaction mixture was heated to 70°C for 2 hr and
cooled. The polymer was precipitated in acetone, redissolved in
15 hot water and precipitated again in acetone. Finally it was
dried under vacuum for 12 hr at 60°C. 5-10% w/v solution of this
polymer in PBS was mixed with the UV photoinitiator and
polymerized using long wavelength UV light to make microspheres
200-1,000 microns in size.

20 These microspheres were stable to autoclaving in water,
which indicates that the gel is covalently cross-linked. The gel
is extremely elastic. This macromer, PVA multiacrylate, may be
used to increase the crosslinking density in PEG diacrylate gels,
with corresponding changes in mechanical and permeability
25 properties. This approach could be pursued with any number of
water-soluble polymers chemically modified with
photopolymerizable groups, for example with water-soluble
polymers chosen from polyvinylpyrrolidone, polyethyloxazoline,

5 polyethyleneoxide-polypropyleneoxide copolymers, polysaccharides such as dextran, alginate, hyaluronic acid, chondroitin sulfate, heparin, heparin sulfate, heparan sulfate, guar gum, gellan gum, xanthan gum, carrageenan gum, and proteins, such as albumin, collagen, and gelatin.

10

Example 29

Use of Alternative Photopolymerizable Moieties

Many photopolymerizable groups may be used to enable gelation. To illustrate a typical alternative synthesis, a synthesis for PEG 1K urethane methacrylate is described as follows:

In a 250 ml round bottom flask, 10 g of PEG 1K diol was dissolved in 150 ml benzene. 3.38 g of 2-isocyanatoethylmethacrylate and 20 μ l of dibutyltindilaurate were slowly introduced into the flask. The reaction was refluxed for 6 hours, cooled and poured into 1000 ml hexane. The precipitate was then filtered and dried under vacuum at 60°C for 24 hours.

In this case, a methacrylate free radical polymerizable group was attached to the polymer via a urethane linkage, rather than an ester link as is obtained, e.g. when reacting with aryloxyl chloride.

Example 30**Formation of Alginate-PLL-alginate Microcapsules with
Photopolymerizable Polycations**

Alginate-polylysine-alginate microcapsules are made by adsorbing, or coacervating, a polycation, such as polylysine (PLL), upon a gelled microsphere of alginate. The resulting membrane is held together by charge-charge interactions and thus has limited stability. To increase this stability, the polycation can be made photopolymerizable by the addition of a carbon-carbon double bond, for example. This can be used to increase the stability of the membrane by itself, or to react, for example, with photopolymerizable PEG to enhance biocompatibility.

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To illustrate the synthesis of such a photopolymerizable polycation, 1 g of polyallylamine hydrochloride was weighed in 100 ml glass beaker and dissolved in 10 ml distilled water (DW). The pH of the polymer solution was adjusted to 7 using 0.2 M sodium hydroxide solution. The polymer was then separated by precipitating in a large excess of acetone. It was then redissolved in 10 ml DW and the solution was transferred to 50 ml round bottom flask. 0.2 ml glycidyl methacrylate was slowly introduced into the reaction flask and the reaction mixture was stirred for 48 hours at room temperature. The solution was poured into 200 ml acetone and the precipitate was separated by filtration and dried in vacuum.

5 This macromer is useful in photochemically stabilizing an alginate-PLL-alginate, both in the presence or in the absence of a second polymerizable species such as a PEG diacrylate.

In addition to use in encapsulating cells in materials such as alginate, such photopolymerizable polycations may be 10 useful as a primer or coupling agent to increase polymer adhesion to cells, cell aggregates, tissues and synthetic materials, by virtue of adsorption of the photopolymerizable polymer bonding to the PEG photopolymerizable gel.

Example 31

**15 Cellular Microencapsulation for Evaluation of
Anti-Human Immunodeficiency Virus Drugs In Vivo**

HIV infected or uninfected human T-lymphoblastoid cells can be encapsulated into PEG gels as described for other cells above. These microcapsules can be implanted in a nonhuman animal and then treated with test drugs such as AZT or DDI. After treatment, the microcapsules can be harvested and the encapsulated cells screened for viability and functional normalcy using a fluorescein diacetate/ethidium bromide live/dead assay. Survival of infected cells indicates successful action of the 25 drug. Lack of biocompatibility is a documented problem in this approach to drug evaluations, but the highly biocompatible gels described herein solve this problem.

Example 32

Use of Alternative

Photoinitiator/Photosensitizer Systems

It is possible to initiate photopolymerization with a wide variety of dyes as initiators and a number of electron donors as effective cocatalysts. Table 10 illustrates photopolymerization initiated by several other dyes which have chromophores absorbing at widely different wavelengths. All gelations were carried out using a 23% w/w solution of 18.5 kD PEG tetraacrylate in HEPES buffered saline. These initiating systems compare favorably with conventional thermal initiating systems, as can also be seen from Table 10.

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Table 10

Polymerization Initiation

	INITIATOR	LIGHT SOURCE*	TEMPERATURE °C	APPROXIMATE GEL TIME, (SEC)
10	Eosin Y, 0.00015M, Triethanolamine 0.65M	S1 with UV filter	25	10
	Eosin Y, 000015M; Triethanolamine 0.65M	S4	25	0.1
15	Methylene Blue, 0.00024M; p- toluenesulfonic acid, 0.0048M	S3	25	120
	2,2-dimethoxy-2-phenyl acetophenone 900 ppm	S2	25	8
20	Potassium Persulfate 0.0168M	-	75	180
	Potassium Persulfate 0.0168M; tetramethyl ethylene-diamine 0. 039M	-	25	120
25	Tetramethyl ethylene- diamine 0.039M; Riboflavin 0.00047M	S1 with UV filter	25	300

* LIST OF LIGHT SOURCES USED

	CODE	SOURCE
30	S1	Mercury lamp, LEITZ WETZLER Type 307-148.002, 100W
	S2	Black Ray longwave UV lamp, model B-100A W/FLOOD
	S3	MELLES GRIOT He-Ne laser, 10mW output, $\lambda=632$ nm
	S4	American laser corporation, argon ion laser, model 909BP-15-01001; $\lambda=488$ and 514 nm

35 Numerous other dyes can be used for photopolymerization. These dyes include but are not limited to Erythrosin, phloxime, rose bengal, thionine, camphorquinone,

ethyl eosin, eosin, methylene blue, and riboflavin. Possible cocatalysts that can be used include but are not limited to: N-methyl diethanolamine, N,N-dimethyl benzylamine, triethanolamine, triethylamine, dibenzyl amine, N-benzyl ethanolamine, N-isopropyl benzylamine.

BIODEGRADABLE MACROMERS

Table 11 shows the code names of the various macromers synthesized in or for use in the examples, along with their composition in terms of the molecular weight of the central PEG segment and the degree of polymerization of the degradable comonomer.

5 Table 11: Macromer Molecular Weight and Composition

	PEG molecular weight	Comonomer	D.P. of comonomer per OH group	Polymer weight code
10	20,000	glycolide	15	20KG
	18,500	glycolide	2.5	18.5K
	10,000	glycolide	7	10KG
	6,000	glycolide	5	6KG
	4,000	glycolide	5	4KG
	1,000	glycolide	2	1KG
15	20,000	DL-lactide	10	20KL
	18,500	DL-lactide	10	18.5KL
	10,000	DL-lactide	5	10KL
	6,000	DL-lactide	5	6KL
	1,000	DL-lactide	2	1KL
	600	DL-lactide	2	0.6KL
20	600	DL-lactide + lactide 2;		0.6KLCL
		caprolactone (CL)	CL 1	
	18,500	caprolactone	2.5	18.5KCL
	18,500	-	-	18.5KCO

25 Example 33

Synthesis of Photopolymerized Biodegradable Hydrogels.**PEG-based hydrogels**

PEG-based biodegradable hydrogels are formed by the rapid laser or UV photopolymerization of water soluble macromers. Macromers, in turn, are synthesized by adding glycolic acid oligomers to the end groups of PEG and then capping with acrylic end groups. The PEG portions of the macromers confer water solubility properties, and subsequent polymerization results in cell-nonadhesive hydrogels. Glycolic acid oligomers serve as the hydrolyzable fraction of the polymer network, while acrylic end

5 groups facilitate rapid polymerization and gelation of the macromers.

In preparation for synthesis, glycolide (DuPont) or DL-lactide (Aldrich) was freshly recrystallized from ethyl acetate. PEG oligomers of various molecular weight (Fluka or Polysciences) 10 were dried under vacuum at 110°C prior to use. Acryloyl chloride (Aldrich) was used as received. All other chemicals were of reagent grade and used without further purification.

Macromer synthesis

A 250 ml round bottom flask was flame dried under repeated cycles of vacuum and dry argon. 20 gm of PEG (molecular weight 10,000), 150 ml of xylene and 10 μ gm of stannous octoate were charged into the flask. The flask was heated to 60°C under argon to dissolve the PEG and cooled to room temperature. 1.16 gm of glycolide was added to the flask and the reaction mixture was refluxed for 16 hr. The copolymer was separated on cooling and was recovered by filtration. This copolymer was separated on cooling and recovered by filtration. This copolymer (10K PEG-glycolide) was used directly for subsequent reactions. Other polymers were similarly synthesized using DL-lactide or ϵ -caprolactone in place of glycolide and using PEG of different 25 molecular weights.

Synthesis of photosensitive oligomers (macromers):

19 gm of 10K PEG-glycolide copolymer was dissolved in 150 ml methylene chloride and refluxed with 1 ml acryloyl

5 chloride and 1.2 ml of triethylamine for 12 hr under an argon
atmosphere. The solid triethylamine hydrochloride was separated
by filtration and the polymer was precipitated by adding the
filtrate to a large excess of hexane. The polymer (capped by an
acrylate at both ends) was further purified by repeated
10 dissolution and precipitation in methylene chloride and hexane
respectively.

Table 12 lists certain macromers synthesized. The degree of polymerization of the glycolide chain extender was kept low so that all polymers have approximately 10 ester groups per chain, or about 5 per chain end. When these polymers are photopolymerized, a crosslinked three-dimensional network is obtained. However, each chain segment in the resulting network needs just one ester bond cleaved at either end to "degrade." These ester cleavages enable the chain to dissolve in the surrounding physiological fluid and thereby be removed from the implant site. The resulting hydrolysis products, PEG and glycolic acid, are water soluble and have very low toxicity.

TABLE 12

Macromers Synthesized

Polymer Code	Mol. Wt. Of	%	% ε-	Calculated	Appearance	
	Central PEG	Glycolide	Caprolactone	Mol. Wt. Of		
	Chain	in	in Extremities	Extremities		
	(daltons)	Extremities		(daltons)		
0.4K	400	100	--	580	Viscous liquid	
1KG	1000	100	--	300	Viscous liquid	
10	4KG	4000	100	--	232	White solid
	10KG	10000	100	--	580	White solid
	18.5KG	18500	100	--	1160	Yellow solid
	co18.5KGCL	18500	50	--	580	White solid

Due to the presence of only a few units of glycolic acid per oligomeric chain, the solubility properties of the photocrosslinkable prepolymers are principally determined by the central PEG chain. Solubility of the macromers in water and methylene chloride, both of which are solvents for PEG, is not adversely affected as long as the central PEG segment has a molecular weight of 1,000 daltons or more. Solubility data for the prepolymers synthesized is given in Table 13.

5 Table 13: SOLUBILITY DATA

	Solvent	1KG	4KG	10KG	18.5KG	TMP*
	DMSO	-	■	-	■	■
	Acetone	-	■	■	■	-
	Methanol	-	■	-	■	-
10	Water	-	-	-	-	■
	Hexane	■	■	■	■	■
	Methylene					
	Chloride	-	-	-	-	-
	Cold Xylene	■	■	■	■	-
15	Hot Xylene	-	-	-	-	-
	Benzene	■	■	■	■	-

- Soluble

■ Not Soluble

* Trimethylolpropane glycolide triacrylate

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20 PEG chains with different degrees of polymerization of DL-lactide were synthesized to determine the degree of substitution for which water solubility of the macromers can be retained. The results are shown in Table 14. Beyond about 20% substitution of the hydrophilic PEG chain with hydrophobic DL-lactoyl or acrylate terminals leads to the macromers becoming 25 insoluble in water, though they are still soluble in organic solvents such as methylene chloride.

5 Table 14: Solubility of Macromers

D.P.* of

D.P.* of lactide % extension of Solubility

Ethylene Oxide or glycolide PEG chain in water

	D.P.	% ext.	Solubility
10	420	4	0.1 soluble
	420	10	2.4 soluble
	420	20	4.8 soluble
	420	40	9.5 soluble
	420	80	19 insoluble
15	23	2	8.7 soluble
	23	4	17.4 soluble
	23	10	43.5 insoluble
	23	40	174 insoluble
	5	4	80 insoluble
20	10	4	40 soluble

* degree of polymerization

Photopolymerization

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The macromers can be gelled by photopolymerization using free radical initiators, with the presence of two acrylic double bonds per chain leading to rapid gelation. A 23% w/w solution of various degradable polymers in HEPES buffered saline containing 3 μ l of initiator solution (300 mg/ml of 2,2-dimethoxy-2-phenyl-acetophenone in n-vinyl pyrrolidone) was used. 100 μ l of the solution was placed on a glass coverslip and irradiated with a low intensity long wavelength UV (LWUV) lamp (Blak-Ray, model 3-100A with flood). The times required for gelation to occur were noted and are given below. These times are typically in the range of 10 seconds. This is very significant because these reactions are carried out in air (UV

5 initiated photopolymerizations are slow in air as compared to an inert atmosphere) and using a portable, low powered long wave UV (LWUV) emitting source. Oxygen, which often inhibits free radical reactions by forming species which inhibit propagation, did not seem to slow down the polymerization. Such fast
10 polymerizations are particularly useful in applications requiring *in situ* gelations. This rapid gelation is believed to be due to the formation of micelle-like structures between the relatively hydrophobic polymerizable groups on the macromer, thereby increasing the local concentration of the polymerizable species
15 in aqueous solution and increasing polymerization rates.

Visible laser light is also useful for polymerization. Low intensity and short exposure times make visible laser light virtually harmless to living cells since the radiation is not strongly absorbed in the absence of the proper chromophore. Laser light can also be transported using fiber optics and can be focused to a very small area. Such light can be used for rapid polymerization in highly localized regions; gelation times for selected prepolymers are given in Table 15. In each case, 0.2 ml of a 23% w/v photosensitive oligomer solution is mixed with ethyl eosin (10^{-4} M) and triethanol amine (0.01 to 0.1 M) and the
20 solution is irradiated with an argon ion laser (American argon ion laser model 905 emitting at 514 nm) at a power of 0.2-0.5 W/cm². The beam is expanded to a diameter of 3 mm and the sample
25 is slowly scanned until gelation occurs.

5 Table 15: Gelation Times

Polymer	UV polymerization *	Laser Polymerization**
	gelation time (mean ±S.D.)	gelation time (s)
	(s)	

10	1KG	5.3 ± 4.1	<1
	4KG	14.7 ± 0.5	<1
	6KG	9.3 ± 0.5	<1
	10KG	$18. \pm 0.8$	<1
15	10KL	7.7 ± 0.5	<1
	18KG	23.3 ± 1.2	<1
	20KG	13.3 ± 0.5	<1

* Initiator: 2,2-dimethoxy-2-phenylacetophenone, concentration 900 ppm: 0.2 ml of 23% monomer solution in PBS

** Argon ion laser emitting at 514nm. power 3 W/cm²: ethyloesin, triethanol amine initiating system: 0.2 ml of 23% monomer solution in PBS

Biodegradability

Biodegradation of the resulting polymer network is an important criteria in many biomedical applications. Degradation of poly(glycolic acid and poly(DL-lactic acid) has been well documented in the literature. The degradation mainly takes place through the hydrolysis of the ester bond; the reaction is second order and highly pH dependent. The rate constant at pH 10 is 7 times faster than that at pH 7.2.

Such facile biodegradation is surprising because poly(α -hydroxyacidesters) are hydrophobic and highly insoluble in water. Accessibility of the polymer matrix to the aqueous

5 surrounding is therefore limited. However, because the networks
 are hydrogels which are swollen with water, all the ester
 linkages in the network are in constant contact with water with
 the aqueous surroundings. This results in a uniform bulk
 degradation rather than a surface degradation of these gels.

10 Table 16 gives hydrolysis data for some of these
 networks; times listed are for complete dissolution of 60 mg of
 gel at pH 7.2 and 9.6. As noted, most of the gels dissolve
 within 12 hours at pH 9.6. 18.5K gel dissolves within 2.5 hr at
 pH 9.6 whereas 18.5KCO gel does not dissolve in 3 days,

15 indicating that the lactoyl, glycoloyl, or ϵ -caprolactoyl ester
 moiety is responsible for degradation of these networks. It also
 can be seen that the 18.5KG gel hydrolyzes more rapidly than the
 4KG gel. This may be due to the reduced hydrophilicity and
 higher crosslink density of the latter gel.

20 Table 16: Hydrolysis Data

Oligomer used for gelation	Time taken to dissolve gel at pH 9.6 (h)	Time taken to dissolve gel at pH 7.2 (days)
4KG	6.2	5.5
10KG	12.25	5.5
18.5KG	2.25	>7
18.5KCL	>5 days	>7
18.5KCO	>5 days	>7

5 Characterization of macromers

FTIR spectra of the prepolymers were recorded on a DIGILAB model FTS 15/90. The absorption at 1110 cm⁻¹ (characteristic C-O-C absorption of PEG) shows the presence of PEG segments. The strong 1760 cm⁻¹ absorption shows the presence of glycolic ester. The absence of hydroxyl group absorption around 3400 cm⁻¹ and a weak acrylic double bond absorption at 1590 cm⁻¹ shows the presence of acrylic double bonds at the end groups.

500 MHz proton and 125 MHz carbon-13 spectra were recorded on a GE 500 instrument. The presence of a very strong peak at 4.9 ppm due to CH₂ methylene from the PEG segment, a peak at 5.09 ppm due to the glycolic ester segment and an acrylic proton singlet at 5.8 ppm can be easily seen from proton NMR. The estimated molecular weight of PEG segment and glycolic acid segment for different copolymers is shown in Table 12. The carbonyl peak at 169.39 ppm from glycolic acid and 36.5 ppm peak from methylene carbons from PEG in carbon-13 NMR are consistent with the reported chemical composition of these copolymers.

Differential scanning calorimetry (Perkin Elmer DSC-7) was used to characterize the oligomers for thermal transitions. The oligomers were heated from -40°C to 200°C at a rate of 20°C/min, presumably causing polymerization. The polymer was then cooled to -40°C at a rate of 60°C/min and again heated to 200°C at a rate of 20°C/min. The first scans of biodegradable

5 18.5K PEG glycolide tetraacrylate (18.5KG) oligomer were compared
to that of the non-degradable 18.5K PEG tetraacrylate (18.5KCO)
scan. It was seen that a glass transition appears in the 18.5KG
at -2°C while no such transition exists in the 18.5KCO. A small
melting peak at 140°C was also evident due to the few glycolic
10 acid mers which can crystallize to a limited extent. The melting
peak for PEG is shifted downwards in 18.5KG to 57°C from 60.7°C
'
for 18.5KCO. This is probably due to disturbance of the PEO
crystalline structure due to the presence of the glycolic acid
linkages. In the third cycle, by which time the oligomers have
15 presumably polymerized, the Tg and Tm transitions for the
glycolide segments can no longer be seen, indicating that a
crosslinked network has formed and the glycolic acid segments are
no longer capable of mobility.

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The degree of polymerization (D.P.) of the degradable
segments added to the central water soluble PEG chain was
determined in several cases using ¹H NMR. The experimentally
determined D.P. was seen to be in good agreement with the
calculated number, as shown by Figure 15. Thus, the ring opening
reaction initiated by the PEG hydroxyls proceeds to completion,
25 giving quantitative yields.

Determination of Total Water, Free Water Bound Water

Solutions of various degradable macromers were made as
described above. Gels in the shape of discs were made using a
mold. 400 μ l of solution was used for each disc. The solutions

5 were irradiated for 2 minutes to ensure thorough gelation. The
disc shaped gels were removed and dried under vacuum at 60°C for
2 days. The discs were weighed (W1) and then extracted
repeatedly with chloroform for 1 day. The discs were dried again
and weighed (W2). The gel fraction was calculated as W2/W1.
10 This data appears in Table 17.

Subsequent to extraction, the discs were allowed to
equilibrate with PBS for 6 hours and weighed (W3 after excess
water had been carefully swabbed away). The total water content
was calculated as (W3-W2) X 100/W3. Differential scanning
calorimetry (DSC) was used to determine the amount of free water
that was available in the gels. A scan rate of 20°C/min was used
and the heat capacity for the endotherm for water melting was
measured (H1). The heat capacity of HBS was also measured (H2).
The fraction of free water was calculated as H1/H2. The residual
water was assumed to be bound due to hydrogen bonding with the
PEO segments. The presence of free water in the gels was
indicated. This free water can be expected to help proteins and
enzymes entrapped in such gels in maintaining their native
conformation and reducing deactivation. Thus these gels would
25 appear to be suited for controlled release of biological
micromolecules. The data for gel water content is summarized in
Table 17.

5 Table 17: Hydrogel Water content

Polymer Code	% Free Water	% Bound Water	% Total Water	% Gel Content
10	1KG	68.4	14	82.3±2.6
	4KG	78.0	9.3	87.3±1.8
	6KG	74.8	13.4	88.1±3.3
	10KG	83.7	10.8	94.5±0.5
	10KL	82.0	9.7	91.7±0.5
	18.5KG	71.8	22.3	94.0±0.4
	20KG	79.8	14.8	94.5±0.4

15 Example 34

Use of multifunctional macromers.

30 g of a tetrafunctional water soluble PEG (MW 18,500)

(PEG 18.5k) was dried by dissolving the polymer in benzene and distilling off the water benzene azeotrope. In a glove bag, 20 g of PEG 18.5 k, 1.881 g of glycolide and 15 mg of stannous octoate were charged into a 100 ml round bottom flask. The flask was capped with a vacuum stopcock, placed into a silicone oil bath and connected to a vacuum line. The temperature of the bath was raised to 200°C. The reaction was carried out for 4 hours at

25 200°C and 2 hours at 160°C. The reaction mixture was cooled, dissolved in dichloromethane and the copolymer was precipitated by pouring into an excess of dry ethyl ether. It was redissolved in 200 ml of dichloromethane in a 500 ml round bottom flask

30 cooled to 0°C. To this flask, 0.854 g of triethylamine and 0.514 ml of acryloyl chloride were added under nitrogen atmosphere and the reaction mixture was stirred for 12 h. at 0°C. The triethyl

5 amine hydrochloride was separated by filtration and the copolymer
was recovered from filtrate by precipitating in diethyl ether.
The polymer was dried at 50°C under vacuum for 1 day.

Example 35

Synthesis of a photosensitive macromer containing DL-lactide

10 PEG (MW) 20,000) (PEG 20k) was dried by dissolving in
benzene and distilling off the water benzene azeotrope. In a
glove bag, 32.43 g of PEG 20k, 2.335 g of DL-lactide and 15 mg of
stannous octoate were charged into a 100 ml round bottom flask.
The flask was capped with a vacuum stopcock, placed into a
silicone oil bath and connected to a vacuum line. The
temperature of the bath was raised to 200°C. The reaction was
carried out for 4 hours at 200°C. The reaction mixture was
cooled, dissolved in dichloromethane and the copolymer was
precipitated by pouring into an excess of dry ethyl ether. It
was redissolved in 200 ml of dichloromethane in a 500 ml round
bottom flask cooled to 0°C. To this flask, 0.854 g of
triethylamine and 0.514 ml of acryloyl chloride were added under
nitrogen atmosphere and the reaction mixture was stirred for 12
hours at 0°C. The triethyl amine hydrochloride was separated by
25 filtration and the copolymer was recovered from filtrate by
precipitating in diethyl ether. The polymer was dried at 50°C
under vacuum for 1 day.

Example 36

Synthesis of a Photosensitive Precursor

Containing DL-Lactide and ε-Caprolactone.

PEG (MW 600) (PEG 0.6k) was dried by dissolving in benzene and distilling off the water benzene azeotrope. In a glove bag, 0.973 g of PEG 0.6k, 0.467 g of DL-lactide along with 0.185 g of ε-caprolactone and 15 mg of stannous octoate were charged into a 50 ml round bottom flask. The flask was capped with a vacuum stopcock, placed into a silicone oil bath and connected to a vacuum line. The temperature of the bath was raised to 200°C. The reaction was carried out for 4 hours at 200°C and 2 hours at 160°C. The reaction mixture was cooled, dissolved in dichloromethane and the copolymer was precipitated by pouring into an excess of dry ethyl ether. It was redissolved in 50 ml of dichloromethane in a 250 ml round bottom flask cooled to 0°C. To this flask, 0.854 g of triethylamine and 0.514 ml of acryloyl chloride were added under nitrogen atmosphere and the reaction mixture was stirred for 12 hours at 0°C. The triethyl amine hydrochloride was separated by filtration and the copolymer was recovered from filtrate by precipitating in diethyl ether. The polymer was dried at 50°C under vacuum for 1 day and was a liquid at room temperature.

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Example 37**Selection of dyes for use in photopolymerization**

It is possible to initiate photopolymerization with a wide variety of dyes as initiators and a number of electron donors as effective cocatalysts. Table 18 illustrates 10 photopolymerization initiated by several other dyes which have chromophores absorbing at widely different wavelengths. All gelations were carried out using a 23% w/w solution of 18.5KG in HEPES buffered saline. These initiating systems compare favorably with conventional thermal initiating systems, as can also be seen from Table 18. Other photoinitiators that may be 15 particularly useful are 2-methoxy-2-phenyl acetophenone and camphorquinone.

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5 Table 18: Polymerization Initiation of 18.5KG PEG

	INITIATOR	LIGHT SOURCE*	TEMPERATURE °C	GEL TIME (SEC)
10	Eosin Y, 0.00015M; Triethanolamine 0.65M	S1 with UV filter	25	10
	Eosin Y, 0.00015M; Triethanolamine 0.65M	S4	25	0.1
15	Methylene Blue, 0.00024M; p-toluenesulfonic acid, 0.0048M / 2,2-dimethoxy-2-phenyl acetophenone 900 ppm	S3	25	120
	Potassium persulfate 0.0168M	S2	25	8
20	Potassium Persulfate 0.0168M; tetramethyl ethylene-diamine 0.039M	-	75	180
25	Tetramethyl ethylene- diamine 0.039M; Riboflavin 0.00047M	S1 with UV filter	25	300

*LIST OF LIGHT SOURCES USED

CODE	SOURCE	
S1	Mercury lamp, LEITZ WETSLER Type 307-148.002,	100W
S2	Black Ray longwave UV lamp, model B-100A W/FLOOD	
S3	MELLES GRIOT He-Ne laser, 10mW output, $\lambda=632$ nm	
S4	American laser corporation, argon ion laser, model 909BP-15-01001; $\lambda=488$ and 514 nm	

35 Numerous other dyes can be used for
photopolymerization. These dyes include but are not limited to:
Erythrosin, phloxine, rose bengal, thioneine, camphorquinone,
ethyl eosin, eosin, methylene blue, and riboflavin. The several
possible cocatalysts that can be used include but are not limited
40 to: N-methyl diethanolamine, N,N-dimethyl benzylamine,

5 triethanol amine, triethylamine, dibenzyl amine, N-benzyl
ethanolamine, N-isopropyl benzylamine, and N-vinyl pyrrolidinone.

Example 38

Thermosensitive Biodegradable Gels from N-Isopropyl Acrylamide

Synthesis of low molecular weight polyisopropyl acrylamide

10 N-isopropyl acrylamide (NIPAAm) was recrystallized from
65:35 hexane benzene mixture. Azobisisobutyronitrile (AIBN) was
recrystallized from methanol. 1.5 g of NIPAAm was polymerized
using 3 mg of AIBN and 150 mg of mercaptoethanol in 1:1 acetone
water mixture (24 hours at 65°C). The viscous liquid after
polymerization was purified by dissolving in acetone and
precipitating in diethyl ether. Yield 80%.

This hydroxy terminated low molecular weight
poly(NIPAAm) was used in chain extension reactions using
glycolide and subsequent endcapping reaction using acryloyl
chloride as described in other examples.

1 g of modified poly(NIPAAm) based oligomer and 0.2 g
IKL were dissolved in water at 0°C and polymerized at 0°C using
2-2-dimethoxy-2-phenylacetophenone (900 PPM).

Example 39**In Vitro Degradation**

The gels were extracted as described in Example 32 to remove the unpolymerized macromer fraction fraction and the gels were then placed in 50 mM HEPES buffered saline (0.9% NaCl), pH 7.4 at 37°C. Duplicate samples were periodically removed, washed with fresh HBS and dried at 100°C for 1 day and weighed to determine mass loss in the gel. The compositions of the various gels used were the same as described in the previous examples.

Table 19 shows the extent of degradation of these gels given as percent of mass lost over time. The respective times are given in parenthesis along with the mass loss data.

Table 19: Gel Degradation

1KG	20.1% (1 d), 20.36±0.6 (2d), 21.7± (6d), 28.8±16.6 (10 d) estimated total Degradation time 45 days.
4KG	38.9 (1d), 60.3±4.2 (2d), 78.9 (3d), 99.3±4.7 (6d). Total degradation time 5.5 days.
6KG	18.3±6.8 (1d), 27.4±1.0 (2d), 32.8±11.3 (3d), 104.8±3.2 (5d). total degradation time 4.5 days 10KG 0.6±0.6 (8 hr), 100 (1d). Total degradation time 1 day.
10KL	10.0±4.84 (2d), 6.8±1.7 (3d), 4.5±3.1 (6d), 8.0±0.2 (10d). Total degradation time estimated to be 20 days.
20KG	68.1±4.2 (8hr), 99.7±0.3 (1d). Total degradation time 15 hr.

Example 40**Fibroblast adhesion and spreading**

The *in vitro* response of Human foreskin fibroblast (HFF) cells to photopolymerized gels was evaluated through cell culture on polymer networks. 0.2 ml of monomer solution was UV 10 polymerized on an 18 x 18 mm glass coverslips under sterile conditions. HFF cells were seeded on these gels at a cell density of 1.8×10^4 cells/sq cm of coverslip area in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal calf serum. The gels were incubated for 6 hr at 37°C in a 5% CO₂ environment, at the end of which they were washed twice with phosphate buffered saline (PBS). The adherent cells were fixed 15 using a 2% glutaraldehyde solution in PBS. The gels were examined under a phase contrast microscope at a magnification of 200X, and the number of adherent and spread cells evaluated by examining five fields selected at predetermined locations on the coverslips.

The number of adherent cells is reported in Table 20 along with those for glass control surfaces. Cell adhesion is seen to be dramatically lowered on gel-coated glass.

5 **Table 20: Cell Adhesion**

Surface	Attached Cells/cm ²
glass	13220±3730
18.5KG	250±240
18.5KCL	1170±1020
10 18.5KCO	390±150

Typical photographs of these cells on the 18.5KCL gel surfaces and on control glass surfaces are shown in Figures 16A and 16B. It can be easily seen from Table 20 that these gels are highly resistant to cellular growth. Even the 18.5KCL is still less than 10% of the glass. Cells attached to the glass surface show a flattened and well-spread morphology whereas the few cells that are attached to the gel are rounded and loosely attached. This may result from the fact that hydrated PEG chains have a high motility and have been shown to be effective in minimizing protein adsorption. One of the mechanisms by which cell adhesion is mediated is through the interaction of cell surface receptors with adsorbed cell adhesion proteins. Thus the reduction in overall protein adsorption results in minimal cell adhesion protein adsorption and reduced cell adhesion.

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Example 41**Release of Protein (Bovine Serum Albumin) from Polymers**

1KG was used for this study. This macromer was liquid at room temperature and was used as such. 1 mg of bovine serum albumin (BSA) was added per ml of monomer solution along with 0.9

5 mg/ml of 2,2-dimethoxy-2-phenyl-acetophenone as initiator. The protein was dissolved in the monomer solution and disc shaped gels were made by exposing 0.2 g of macromer mixture to LWUV for 1 min. Two such discs were placed in a flask containing 20 ml of PBS and incubated at 37°C. Two aliquots of 20 μ l each were 10 removed from these flasks periodically and the amount of BSA released was assayed using the Bio-Rad total protein assay. The release profile for BSA is shown in Figure 17A. It can be seen that the release of BSA is relatively steady over more than a month.

15 **Example 42**

Enzyme Release Assay

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Water solubility of the macromers means gelation can be carried out in a non-toxic environment. This makes these materials suitable for intraoperative uses where *in situ* gelation is needed. Since the precursors are water soluble, the gels can be used as drug delivery vehicles for water soluble drugs, especially macromolecular drugs such as enzymes, which would otherwise be denatured and lose their activity. Release of lysosome and tPA from the polymers was used to illustrate the feasibility of using biodegradable hydrogels for controlled release of biomolecules.

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Lysozyme release

The enzyme lysozyme (MW:14,400) is a convenient model for release of a low molecular weight protein from a biodegradable gel. The Biorad total protein assay was used to quantify the enzyme released. The enzyme was dissolved in PBS at 10 a concentration of 20 mg/ml. The monomer PEG-dl-lactic acid-diacrylate was dissolved in PBS to produce a 40% solution. The lysozyme solution was added to the monomer solution to attain a 24% monomer solution. The monomer/lysozyme solution was polymerized under UV in a cylindrical mold, using 30 μ l of the initiator 2,2-dimethoxy-2-phenyl-acetophenone in 1-vinyl-2-pyrrolidone (30 mg/ml) as the initiator. The polymer was cut into 10 equal sized pieces and immersed in 10 ml PBS. Samples of the PBS were withdrawn at intervals and assayed for lysozyme released into the PBS. Lysozyme was released from the PEG-DL-lactic acid-diacrylate gel over an 8 day interval, with the maximum rate of release occurring within the first 2 days, as shown by Figure 17B.

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25Release of recombinant t-PA

Three macromers were used for these studies: 1KL, 4KG, and 18.5KG. The 1KL macromer was liquid at room temperature and was used as such. The second macromer, 4KG, was used as a 75% w/w solution in PBS. The third composition was a mixture of equal parts of 1KL and a 50% w/w solution of 18.5KG. 3.37 mg of tissue plasminogen activator (single chain, recombinant, M.W.

5 71,000) was added per gram of macromer solution along with 0.9
mg/ml of 2,2 dimethoxy 2 phenyl acetophenone as initiator. The
protein was dissolved with the macromer and disc shaped gels were
made by exposing 0.2 g of macromer mixture to LWUV for 1 minute.
Two such discs were rinsed with PBS, placed in a flask containing
10 5 ml of PBS and incubated at 37°C. Two aliquots of 100 μ l each
were removed from these flasks periodically and the amount of
active t-PA released was assayed using a chromogenic substrate
assay (Kabi-vitrum). The release profiles from the 1K lactide
gels, 4K glycolide gels, and the 50/50 1K glycolide/18.5K
glycolide are shown in Figures 18A - 18C. Fully active tPA can
be released for periods up to at least two months.

15 By selecting an appropriate formulation, the release
rate can be tailored for a particular application. It is also
possible to combine formulations with different molecular weights
so as to synergistically achieve appropriate attributes in
release and mechanical characteristics.

20 For prevention of postoperative adhesions, in addition
to the barrier effect of the gels, the gels can be loaded with a
fibrinolytic agent to lyse incipient filmy adhesions which escape
25 the barrier effect. This further enhances the efficacy of
biodegradable gels in adhesion prevention.

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Example 43**Toxicity of Polymers and Commercial Adhesives**

To evaluate the toxicity of *in situ* polymerization of the macromer solutions described herein, as compared to commercial adhesives, 100 μ l of 18.5KCO prepolymer solution was placed on the right lobe of a rat liver and gelled by exposing it to LWUV for 15 sec; similarly, a few drops of a n-butyl cyanoacrylate based glue were placed on the left lobe. The liver was excised after a week, fixed in 10% neutral buffered formalin, blocked in paraffin, sectioned and stained using hematoxylin and eosin.

No adverse tissue reaction was evident on the surface of the lobe exposed to the biodegradable gel. No inflammatory reaction to the polymerization process can be seen. The epithelium looks normal, with no foreign body reaction.

In comparison, the lobe exposed to cyanoacrylate glue shows extensive tissue necrosis and scarring with 10-30 cell deep necrotic tissue. Fibrosis is evident in the necrotic portions close to underlying normal tissue.

Example 44

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Prevention of Post-Surgical Adhesions with Photopolymerized Biodegradable Polymer

A viscous sterile 23% solution in phosphate buffered saline (8.0 g/l NaCl, 0.201 g/l KCl, 0.611 g/l Na₂HPO₄, 0.191 g/l

5 KH₂PO₄, pH 7.4) of polyethylene glycol (M.W. 18,500) which has
been chain extended on both ends with a short polyglycolide
repeat unit (average number of glycolidyl residues: 10 on each
end) and which has been subsequently terminated with an acrylate
group was prepared. Initiator needed for the crosslinking
10 reaction, 2,2-dimethoxy-2-phenyl acetophenone, was added to the
macromer solution to achieve an initiator concentration of 900
ppm. A 30 second exposure to a long wave UV lamp (Blak Ray) is
sufficient to cause polymerization.

Animal models evaluated

15 Animal models evaluated included a rat cecum model and
a rabbit uterine horn model. In the rat cecum mode, 6 out of 7
animals treated with the macromer solution showed no adhesions
whatsoever, while untreated animals showed consistent dense
adhesion formation. In the rabbit uterine horn model, a
significant ($p<0.01$) reduction in adhesion formation was seen in
the animals treated with the gel. Studies conducted in rats
using only the ungelled viscous precursor solution (no LWUV)
failed to prevent the formation of adhesions.

Rat cecum model

25 Twenty-one Sprague Dawley male rats having an average
weight of 250 gm were divided into three groups for treatment and
two for controls. The abdomen was shaved and prepared with a
betadine solution. A midline incision was made under Equithesin
anesthesia. The cecum was located and 4 to 5 scrapes were made

5 on a region about 2 x 1 cm on one side of the cecum, using a 4 x
4 in gauze pad to produce serosal injury and punctate bleeding.
The abdominal incisions in these animals were closed using a
continuous 4-0 silk suture for the musculoperitoneal layer and
7.5 mm stainless steel staples for the cutaneous layer. A
10 topical antibiotic was applied at the incision site.

The first group consisted of 7 animals serving as
controls without treatment, to confirm the validity of the model.
The second group served as a control with the application of the
precursor but without photopolymerization to form the hydrogel.
15 After induction of the cecal injury, about 0.25 ml of the
precursor solution was applied to the injury site using a pipet.
The abdominal incision was then closed as above.

The third group served as the gel treatment group and
was prepared as the second group except that the precursor film
was exposed to a LWUV lamp for 45 seconds to cause gelation.
Both the obverse and reverse sides of the cecum were similarly
treated with precursor and light. No attempt was made to dry the
surface of the tissue, to remove blood, or to irrigate the area
prior to treatment.

25 The animals were sacrificed at the end of two weeks by
CO₂ asphyxiation. The incisions were reopened and adhesions were
scored for location, extent, and tenacity. The extent of
adhesions was reported as a percentage of the traumatized area of
the cecum which forms adhesions with adnexal organs or the

5 peritoneal wall. Tenacity of the adhesions was scored on a scale from 0 to 4: no adhesions - grade 0; tentative transparent adhesions which frequently separate on their own - grade 1; adhesions that give some resistance but can be separated by hand - grade 2; adhesions that require blunt instrument dissection to separate - grade 3; and dense thick adhesions which require sharp instrument dissection in the plane of the adhesion to separate -
, grade 4.

Rat cecum model results

The control group without treatment shows consistently dense and extensive adhesions. The extent of abraded area covered with adhesions was seen to be $73 \pm 21\%$ (mean \pm S.D., n=7). The severity of adhesions was grade 3.5 ± 0.4 . Most of the adhesions were dense and fibrous, involving the cecum with itself, with the peritoneal wall and with other organs such as the liver, small intestine, and large intestine. Frequently the mesentery was seen to be involved in adhesions. In the control group with the application of precursor solution but without gelation by exposure to the LWUV lamp, the extent of adhesion was $60 \pm 24\%$ (n=7), and the severity of adhesions was 3.1 ± 0.4 . In the gel treated group, the cecum was seen to be completely free of adhesions in 6 out of 7 animals. In one case, a grade 2 adhesion was seen with the mesentery over 10% of the area and a grade 2.5 adhesion was seen over 15% of the area, bridging the cecum to the sutures on the site of the incision in the

5 peritoneal wall. The overall adhesion extent for the group was
4%, and the overall severity was 0.32. No evidence of residual
gel was visible, the gel presumably having degraded within the
prior two weeks. The cecum appeared whitish with a fibrous layer
on the surface in the control group, but the tissue appeared
10 healthy and normal in animals treated with the gel.

Rabbit uterine horn model

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15 Eight sexually mature female New Zealand rabbits
between 2 and 3 kg in weight were prepared for surgery. A
midline incision was made in the lower abdominal region under
Rompun, Ketamine, and Acepromazine anesthesia. The uterine horns
were located and the vasculature to both horns was systematically
cauterized to induce an ischemic injury. One animal was rejected
from the study due to immature uterine horns. Seven rabbits were
selected for the treatment with only the photopolymerizable
hydrogel and two animals were selected for evaluating the
combined efficacy of the hydrogel with a fibrinolytic agent,
tissue plasminogen activator (tPA). 5 mg of tPA/ml macromer
solution was used in the latter case. After cauterization,
macromer solutions (0.5 ml) were applied along the horn and
25 allowed to coat the surface where the cauterization injury had
been induced. After uniform application of the solution was
complete, the horns were exposed to a LWUV lamp for 1 min to
induce gelation. The procedure was repeated on the reverse side
of the horns. The incisions were then closed using a continuous

5 2-0 Vicryl (Ethicon) suture for the musculoperitoneal layer and a
0 Vicryl (Ethicon) suture for the cutaneous layer. No
prophylactic antibiotics were administered. No postoperative
complications or infections were observed. Five animals were
used in the control group. The ischemic injury was made as
10 described and the incision was closed without the application of
the precursor; all techniques were identical between the
/ treatment group and the control group.

Controls were used where the same animal model was
subjected to surgery without application of the macromer; all
15 surgical techniques were identical between the treatment group
and the historical controls.

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The rabbits were reoperated under Ketamine anesthesia
at the end of two weeks to evaluate adhesion formation; they were
sacrificed by intracardiac KCl injection. Adhesion formation was
evaluated for extent and tenacity. Extent of adhesion formation
was evaluated by measuring the length of the uterine horn that
formed adhesions with itself or with the peritoneal wall or other
organs. Tenacity of adhesion was classified as either filmy or
fibrous. Filmy adhesions were usually transparent, less strong,
25 and could be freed by hand. The fibrous adhesions were dense,
whitish, and usually required sharp instrument dissection to be
freed. In cases where only a single filmy adhesion band was
evident, a score of 5% was assigned.

5 Typical samples of the horn were excised for histology
and were fixed in a 10% neutral buffered formalin solution.
Paraffin sections of the samples were stained using hematoxylin
and eosin.

Rabbit uterine horn model results

10 The adhesion score is the % of affected area occupied
by the adhesions, with grading of each as being filmy or fibrous.
15 Distorted horn anatomies were observed in control animals. The
mean score in the control group was $50 \pm 15\%$ of the affected area
of the horn being occupied by adhesions with 10% of these being
filmy and 90% fibrous. Distorted horn anatomies were observed,
as can be seen from Figure 19A which presents a superior view of
the uterine horn in an animal used as a control, which showed
adhesions over 66% of the horn surface. The group of animals
treated only with the photopolymerized macromer showed an
adhesion score of $13 \pm 11.4\%$ ($n=10$). Of these, 4 animals showed
less than 5% adhesions with only an occasional filmy band
visible.

20 The animals treated with photopolymerized gel
containing tPA showed further improved results over the "gel
only" animals. One animals showed a filmy band on both the right
and left horn. They were assigned a score of 5% with a total
score of 10%. The other animal did not show any adhesions at
all. Thus the total score for these animals was $5 \pm 5\%$.

5 Figure 19B shows normal horn anatomy in a typical horn
which has undergone gel treatment. Adhesions are filmy in all
cases and no dense bands are seen. No traces of the remaining
gel could be observed. Typical samples of horns showing filmy
adhesions showed some fibrous tissue with a 6-15 cell thick layer
10 of fibroblasts showing some collagen fibrils but no formation of
dense collagen fibers. The horns showing no adhesions
 occasionally showed a 1-4 cell thick layer of fibroblasts, but
mostly a normal epithelium with no evidence of inflammatory
cells.

This same procedure was slightly modified as described below as a better mode of using the polymers to prevent postoperative adhesions using the rat uterine horn model.

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Female rats were anesthetized with pentobarbital (50 mg/kg, intraperitoneally), and a midline laparotomy was performed. The uterine horns were exposed, and the vasculature in the arcade feeding the horns was systematically cauterized using bipolar cautery; the most proximal and most distal large vessel on each horn were not cauterized. Following this, the antimesenteric surface of each horn was cauterized at two 1 mm diameter spots on each horn, each separated by a 2 cm distance, the pair centered along the length of each horn. Following injury, 0.5 ml of macromer solution was applied per horn and was gelled by exposure to long wavelength ultraviolet light (365 nm, approximately 20 mW/cm²) for 15 sec per surface on the front side.

5 and on the back side each. The uterus was replaced in the peritoneal cavity, and the musculoperitoneal and skin layers were closed.

The macromer consisted of a PEG chain of MW 8,000 daltons, extended on both sides with a lactic acid oligomer of an 10 average degree of polymerization of 5 lactidyl groups, and further acrylated nominally at both ends by reaction with acryloyl chloride. In one batch, Batch A, the degree of acrylation was determined by NMR to be approximately 75%, and in another, Batch B, it was determined to be greater than 15 approximately 95%. The macromer was dissolved in saline at a specified concentration, and the initiation system used was 2,2-dimethoxy-2-phenyl acetophenone from a stock solution in N-vinyl pyrrolidinone, the final concentration of 2,2-dimethoxy-2-phenyl acetophenone being 900 ppm and the final concentration of N-vinyl pyrrolidinone being 0.15%.

In one set of experiments, macromer from Batch A was applied in varying concentrations, and adhesions were scored at 7 days postoperatively. Scoring was performed by two means. The length of the horns involved in adhesions was measured with a 25 ruler, and the fraction of the total length was calculated. The nature of the adhesions was also scored on a subjective scale, 0 being no adhesions, 1 being filmy adhesions that are easily separated by hand, and 2 being dense adhesions that can only be separated by sharp instrument dissection. Furthermore, one of

5 the samples contained tissue-plasminogen activator (t-PA), which
is known to reduce adhesions, at a concentration of 0.5 mg/ml
(0.5%) macromer solution. The results are shown in Table 21 for
macromer batch A and batch B.

In a third set of experiments, adhesions were formed in
10 female rats as described above, and the adhesions were surgically
lysed 7 days after the initial surgery. The extent and grade of
adhesions was scored during lysis. The animals were divided into
two groups, and one group was treated with macromer from Batch B
at a concentration of 10%. The results are shown in Table 21 as
15 batch B, 10%.

5 Table 21: Reduction of Adhesions with Polymer.

	Concentration	Extent of adhesions	Grade of adhesions	Number of Animals	macromer
		% (S.D.)	(0-2)	-	
<u>Polymer A</u>					
10	15%	24.6 (3.1)	1.1 (0.1)	7	
	20%	33.6 (9.8)	1.2 (0.3)	7	
	25%	37.5 (11.1)	1.2 (0.1)	7	
	30%	54.2 (12.0)	1.6 (0.4)	6	
15	20% + t-PA	18.3 (6.4)	1.1 (0.1)	6	
	Control (saline)	72.6 (18.7)	1.5 (0.2)	7	

Polymer B

20	5%	22.1 (4.2)	1.2 (0.1)	7	
	10%	10.0 (5.1)	1.0 (0)	7	
	15%	17.8 (5.7)	1.0 (0)	7	
	20%	26.3 (11.4)	1.4 (0.2)	7	
	Control (saline)	75.9 (4.4)	1.8 (0.3)	7	

Polymer B. 10%

25	Scoring performed at:	group that became:			
	time of lysis	Controls	85.9 (9.7)	1.8 (0.1)	7
	Time of lysis	Treatment	79.4 (6.8)	1.7 (0.2)	7
30	7 days post-lysis	Controls	78.8 (11.3)	1.8 (0.1)	7
	7 days post-lysis	Treatment	28.2 (5.1)	1.0 (0)	7

The above results illustrate that the photopolymerized macromer can reduce or prevent post operative adhesions in both primary adhesions and adhesiolysis models, and moreover that the gel can be used to locally release a drug to exert a combined beneficial effect.

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Example 45**Nerve anastomosis**

The sciatic nerve of a rat was aseptically severed using a scalpel and allowed to pull apart. The two ends of the nerve were reopposed using sterile forceps, and a 50% solution in buffer of polymer 1KL, a macromer made from PEG 1K with lactide chain extension and acrylate termination, with 0.1% 2,2-dimethoxy-2-phenoxy acetophenone was applied to the nerve stumps. The affected area was illuminated with a 100 W LWUV lamp for 60 seconds, and an adhesive bond was observed to form between the proximal and distal nerve stumps.

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To ensure the biocompatibility of the applied material with the nerve tissue, the same solution of macromer was applied to nonsevered rat sciatic nerves, and the area of the incision was closed using standard small animal surgical technique. The area was reopened at 1 hour or 24 hour postoperatively, and the affected area of the nerve was removed en block and prepared for transmission electron microscopy. No morphological differences were observable between the treated nerves at either time point as compared to control rat sciatic nerves that were otherwise nonmanipulated, even though they had been traumatized and manipulated.

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Example 46**Evaluation of PEG Based Degradable Gels as Tissue Adhesives**

Abdominal muscle flaps from female New Zealand white rabbits were excised and cut into strips 1 cm X 5 cm. The flaps were approximately 0.5 to 0.8 cm thick. A lap joint, 1 cm X 1 cm, was made using two such flaps. Two different compositions, 0.6KL and 1 KL, were evaluated on these tissues. Both these compositions were viscous liquids and were used without further dilution. 125 μ l of ethyl eosin solution in N-vinyl pyrrolidone (20 mg/ml) along with 50 μ l of triethanolamine was added to each ml of the adhesive solution. 100 μ l of adhesive solution was applied to each of the overlapping flaps. The lap joint was then irradiated by scanning with a 2 W argon ion laser for 30 sec from each side. The strength of the resulting joints was evaluated by measuring the force required to shear the lap joint. One end of the lap joint was clamped and an increasing load was applied to the other end, while holding the joint was clamped and an increasing load was applied to the other end, while holding the joint horizontally until it failed. Four joints were tested for each composition. The 1KL joints had a strength of 6.6 ± 1.0 KPa (mean \pm S.D.), while the 0.6KL joints had a strength of 11.4 ± 2.9 KPa. It is significant to note that it was possible to achieve photopolymerization and reasonable joint strength despite the 6-8 mm thickness of tissue. A spectrophotometric estimate

5 using 514 nm light showed less than 1% transmission through such
muscle tissue.

Example 47

Coupling of Photopolymerizable Groups to Proteins (Albumin)

PEG (M.W. 2,000) monoacrylate (5g) was dissolved in 20
10 ml dichloromethane. Triethyl amine (0.523 g) and 2,2,2-
' trifluoroethanesulfonyl chloride (tresyl chloride) (0.017 g) were
added and the reaction was allowed to proceed for 3 hours at 0°C
under nitrogen atmosphere. The reaction mixture was then
filtered and the dichloromethane evaporated to dryness. The
residue was redissolved in a small amount of dichloromethane and
precipitated in diethyl ether. The polymer was then filtered and
dried under vacuum for 10 hours and used directly in the
subsequent reaction with albumin.

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1 g of bovine serum albumin was dissolved in 200 ml of sodium bicarbonate buffer at pH 9. Tresyl activated PEG monoacrylate (5 g) was added and the reaction was stirred for 24 hours at 25°C. Albumin was separated by pouring the reaction mixture into acetone. It was further purified by dialysis using a 15,000 daltons cutoff dialysis membrane. A 10% w/v solution of the PEG acrylated albumin could be photopolymerized with long wave UV radiation using 0.9 mg/ml of 2,2 dimethoxy 2 phenylacetophenone as the initiator. In this gel the degradable segment is the protein albumin.

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Example 48**Modification of Polysaccharides (Hyaluronic Acid)**

In a dry 250 ml round bottom flask, 10 grams of PEG 400 monomethacrylate was dissolved in 100 ml dry dioxane, to which 4.053 g of carbonyl diimidazole (CDI) was slowly introduced under nitrogen atmosphere and the flask was heated to 50°C for 6 h. Thereafter the solvent was evaporated under vacuum and the CDI activated PEG monomer was purified by dissolving in dichloromethane and precipitating in ether twice.

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1 g of hyaluronic acid, 5 g of CDI activated PEG 400 monoacrylate were dissolved in 200 ml sodium borate buffer (pH 8.5) and the solution was stirred for 24 hours. It was then dialyzed using a 15,000 dalton cutoff dialysis membrane to remove unreacted PEG. A 10% w/v solution of the acrylated hyaluronic acid was photopolymerized with long wave UV radiation, using 0.9 mg/ml of 2,2-dimethoxy-2-phenylacetophenone as the initiator. In this gel, the degradable region is hyaluronic acid.

Example 49**PEG Chain Extended with Polyorthocarbonates and Capped****With Urethane Methacrylate**

25 3, 9-bis(methylene) 2,4,8,10-tetraoxaspiro [5,5] undecane (1g) and polyethylene glycol (molecular weight, 1,000, 7.059 g) were weighed into a 250 ml Schlenk tube under dry nitrogen atmosphere in a glove bag. 50 ml of dry tetrahydrofuran

5 was introduced under nitrogen atmosphere and reaction mixture was
stirred for 6 hours at 50°C. This is a typical step growth
reaction with a disturbed stoichiometry, resulting in low
molecular weight polyorthocarbonate with terminal hydroxy
groups. The oligomer was separated by precipitating in hexane
10 and dried under vacuum. 5 g of oligomer was redissolved in dry
THF to which 20 µl of dibutyltindilaurate and 2 ml of 2-
isocyanatoethyl methacrylate were slowly introduced and
temperature was raised to 50°C. It was held there for 6 hours
and cooled. The product was separated by precipitation in
15 hexane. In this gel, the degradable region is a
polyorthocarbonate.

Example 50

Microencapsulation of Animal Cells

A 23% w/w solution of 18.5KG in HEPES buffered saline
20 (5 ml) was used to resuspend 10⁶ CEM-SS cells. Ethyl eosin (10⁻⁴
M) was used as a solution in N-vinyl pyrrolidone as the initiator
and triethanolamine (0.01 M) was used as the coinitiator. The
solution was then exposed through a coextrusion apparatus to an
argon ion laser (514 nm, 2 Watts). The coextrusion apparatus had
25 mineral oil as the fluid flowing annularly (flow rate 4 ml/min)
around an extruding stream of the precursor cell suspension (flow
rate 0.5 ml/min). The microdroplets gelled rapidly on being
exposed to the laser light and were collected in a container

5 containing PBS. The oil separated from the aqueous phase and the
microspheres could be collected in the PBS below. The
microspheres formed were thoroughly washed with PBS buffer to
remove unreacted monomer and residual initiator. The size and
shape of microspheres was dependent on extrusion rate and
10 extruding capillary diameter (18 Ga to 25 Ga). The
polymerization times were dependent on initiator concentration
(ethyl eosin 5 μ M to 0.5 mM, vinyl pyrrolidone (0.001% to 0.1%),
and triethanolamine (5 mM to 0.1 M), laser power (120 mW to 2W),
and monomer concentration (>10%w/v). Spheres prepared using this
15 method had a diameter from 500 μ m to 1,200 μ m. The
polymerizations were carried out at physiological pH in the
presence of air. This is significant since radical
polymerizations may be affected by the presence of oxygen. Cell
viability subsequent to encapsulation was checked by trypan blue
exclusion assay and the encapsulated cells were found to be more
than 95% viable after encapsulation.

Example 51

**Various Formulations for the Prevention of
Post Operative Adhesions**

25 The utility of PEG-oligo(α -hydroxy acid) diacrylates
and tetraacrylates to prevent postoperative adhesions was
evaluated in the rabbit uterine horn model as described above.
The following polymers were synthesized, as described above: PEG

5 6K lactide diacrylate (6KL), PEG 10K lactide diacrylate (10KL).
PEG 18.5K lactide (18.5KL), PEG 20K lactide (20KL). Solutions
with 24% polymer in PBS with 900 ppm 2,2-dimethoxy-2-phenyl
acetophenone, were prepared as described above. The solutions
were applied to the uterine horn after cautery of the vascular
10 arcade and illuminated with a 365 nm LWUV lamp, as described
above. In one formulation, 18.5KL, 5 mg t-PA was mixed into the
solution before application. Controls consisted of animals
manipulated and cauterized but not treated with macromer
solution. Measurement was performed on the 14th ± 1 day. Extent
15 of adhesion was estimated from the fraction of the horn that was
involved in adhesions, and the tenacity of adhesions was scored
as 0, no adhesions; 1, filmy adhesions that offer no resistance
to dissection; 2, fibrous adhesions that are dissectable by hand;
3, fibrous adhesions that are dissectable by blunt instruments;
20 and 4, fibrous adhesions that are dissectable by sharp
instruments. The results were as follows, where the extent of
adhesions and the tenacity of the adhesions are shown.

5 Table 22: Efficacy of Polymer in Preventing Adhesions.

	Formulation	Number of animals	Extent, %, ± S.D.	Tenacity, 0-4 ± S.D.
10	6KL	7	0.9 ± 1.7	0.9 ± 0.7
	10KL	7	0 ± 0	0 ± 0
	20KL	6	4.4 ± 5.0	0.9 ± 0.7
	18.5KL	7	8.9 ± 13.1	1.6 ± 1.3
	t-PA			
	Control	7	35 ± 22	3.3 ± 0.6

Example 52

15 Polymerization of Ultrathin Layers of Polymer on the Surface
of Blood Vessels to Reduce Thrombosis After Vessel Injury

Blood vessels were harvested from rats and were rinsed free of blood. The endothelium of the vessel were removed by inserting a wooden dowel and rotating the vessel over the dowel. One vessel was used as a control, and was exposed to flowing blood as described below without further modification. Another vessel was treated first by exposure to eosin Y at 1 mM in saline, then rinsed in HEPES buffered saline, then filled with a solution of PEG-MA, PEG 10K with acrylate end-capped oligomers of DL lactide, containing triethanolamine (TEA) (100 mM) and N-vinylpyrrolidone (VP) (0.15%) and then illuminated by exposure to an argon ion laser at 0.5 W/cm² for 15 sec. The nonpolymerized prepolymer mixture in the lumen of the vessel was rinsed away with saline. Human blood was collected from the antecubital vein and was anticoagulated with heparin at 2 units/ml. This blood

5 was perfused through each vessel by a syringe pump at a flow rate corresponding to a wall shear rate of approximately 200/s for 7 min. The vessel was then superficially rinsed in saline and fixed in formaldehyde.

The treated vessel did not appear colored or different
10 in color after perfusion compared to its color before perfusion,
while the untreated control vessel appeared blood red. Thin
segments of each vessel were cut from each vessel, were mounted
on end, and were examined by environmental scanning electron
microscopy (ESEM). ESEM is performed on hydrated samples in
relatively low vacuum. This permits the visualization of the
polymer film coating in the swollen and wet state. This is
important to obtain measurements that may be readily interpreted,
since the polymer film is approximately 95% water. A high degree
of thrombosis was readily observed in the control vessel. The
lumen of this vessel was narrowed to less than one-third its
diameter pre-perfusion by the accumulation of thrombus, as shown
in Figure 20A. By contrast, no thrombus could be observed in the
lumen of the treated vessel, as shown in Figure 20B. A higher
magnification of the vessel wall demonstrated no adherent
25 thrombus. A still higher magnification shows a white structure
which is the polymer film, which is different in contrast from
the tissue due to differential charging under the electron beam
of the ESEM. The film may be seen to be precisely conformed to
the shape of the vessel and be approximately 5 - 8 μm thick.

5 The region of polymerization was restricted to the
neighborhood of the blood vessel wall surface. The
photosensitive dye was adsorbed to the vessel wall. Unbound dye
was rinsed away. The entire lumen was filled with prepolymer,
but upon illumination the gel formation was restricted to the
10 vessel wall where the dye and the prepolymer meet. This
interfacial polymerization process can be conducted to produce
surface adherent layers that vary in thickness from less than 7
 μm to more than 500 μm .

15 The above procedure was performed in 8 control rat
arteries, and 8 treated arteries, with equivalent light
microscopic histological results as described above. As
demonstrated by this study, PEG prepolymers can be polymerized
upon the luminal surface of blood vessels. The immediate effect
of this modification is to reduce the thrombogenicity of an
injured blood vessel surface. This has clear utility in
improving the outcome of balloon angioplasty by reducing the
thrombogenicity of the vessel and lesion injured by balloon
dilation. Another effect of this modification is to reduce
smooth muscle cell hyperplasia. This may be expected for two
20 reasons. First, platelets contain a potent growth factor,
platelet-derived growth factor (PDGF), thought to be involved in
post-angioplasty hyperplasia. The interruption of the delivery
of PDGF itself poses a pharmacological intervention, in that a
"drug" that would have been delivered by the platelets would be

5 prevented from being delivered. Thrombosis results in the generation of thrombin, which is a known smooth muscle cell mitogen. The interruption of thrombin generation and delivery to the vessel wall also poses a pharmacological intervention. There are other growth factors soluble in plasma which are known to be
10 smooth muscle cell mitogens. The interruption of thrombin generation and delivery to the vessel wall also poses a pharmacological intervention. Moreover, there are other growth factors soluble in plasma which are known to be smooth muscle cell mitogens. The gel layer is known to present a permselective barrier on the surface of the tissue, and thus the gel layer may reasonably be expected to reduce hyperplasia after angioplasty.
15 The inhibition of thrombosis upon the vessel wall may also reduce the incidence of abrupt reclosure and vasospasm, both of which occur sometimes following vascular intervention.

20 **Example 53**

Interfacial Polymerization of Macromers

Inside Blood Vessels to Prevent Thrombosis

Macromer solutions were polymerized interfacially within previously injured blood vessels *in vivo* to prevent
25 thrombosis. The carotid artery was exposed, and a polyethylene tube (PE-10) was used to cannulate the exterior carotid artery. The artery was clamped with fine arterial clamps proximal to the interior/exterior carotid artery bifurcation and approximately 2

5 cm distal to the bifurcation. A 1 ml tuberculin syringe was used to rinse the blood from the lumen of the isolated zone by filling and emptying the vessel zone. The vessel was injured by crushing using a hemostat. The isolated zone was filled with a 10 mM solution of eosin Y for 2 minutes, after which it was rinsed and
10 filled with a 20% solution of a macromer in saline with 0.1 mM triethanolamine and 0.15% N-vinyl pyrrolidinone. The macromer consisted of a PEG chain of MW 8,000 daltons, extended on both sides with a lactic acid oligomer of an average degree of polymerization of 5 lactidyl groups, and further acrylated
15 nominally at both ends by reaction with acryloyl chloride. The vessel was illuminated transmurally using an argon ion laser (514 nm) at an intensity of approximately 1 mW/cm² for 5 seconds.
Following this, the cannula was removed from the exterior carotid artery and the artery was ligated at the bifurcation. The arterial clamps were removed to permit the resumption of blood flow. Perfusion was allowed for 20 minutes, following which the vessel were again isolated, removed from the body, gently rinsed, fixed, and prepared for light microscopic histological analysis.
Using the naked eye, the crushed segments in control animals,
25 which lacked illumination, were red, indicating internal thrombus with entrapped red blood cells. By contrast, no redness was observed at the site of the crush injury in the treated vessels. Histology showed extensive thrombus, fibrin, and entrapped red blood cells in the non-treated vessels. By contrast, no thrombus

5 or fibrin or entrapped red blood cells were observed in the
treated vessels. The procedure was conducted in four control
animals and three treated animals.

This example demonstrates that the polymerization can be carried out *in situ* in the living animal, that the polymer coating remains adherent to the vessel wall during arterial blood flow, and that the polymer coating can prevent thrombosis *in vivo* in non-anticoagulated animals. This approach to treatment has clear benefits in preventing abrupt reclosure, vasospasm, and restenosis after intravascular interventional procedures.